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Study of agronomical and postharvest factors influencing qualitative and nutraceutical traits on blood orange and pomegranate fruits

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ABSTRACT

The awareness of consumers on the importance of food safety and of potential benefits of many fruit and vegetable derived products are, more and more, driving the interest of research institutes and food industries to deepen the knowledge on the quality of raw materials for fresh or processed use or to design food products enriched with nutraceutical substances.

Several factors influence composition and quality of food products in pre- and post-harvest stages, such as cultivar and rootstock, agronomical techniques and storage conditions. The possibility to enhance the synthesis of some chemical compounds, in particular nutraceuticals (flavonoids, such as phenols and anthocyanins) is an important strategy in order to obtain foods with high functional activity.

The overall aim of the PhD thesis is the evaluation of agronomical and postharvest factors that can influence the qualitative and nutraceutical traits of two important fruit products i.e. blood oranges and pomegranates. These fruits are characterized by a high anthocyanin content greatly appreciated by the consumers for their nutraceutical properties.

The influence of several rootstocks on yield precocity and fruit quality and the effect of postharvest treatments on qualitative parameters were mainly considered in the case of blood oranges.

As concerning pomegranate the investigation was focused on nutraceutical and physicochemical evolution observed in different studies regarding the comparison of international

cultivars grown in Italy and Spain and the characterization of several Sicilian pomegranate accessions.
On the whole, the results are interesting for their contribution to the comprehension of the many factors, from varietal choice up to fruit postharvest management, affecting the qualitative profiles of the products with a special emphasis on those compounds valuable for their nutraceutical properties.

SOMMARIO

La crescente domanda da parte del consumatore di cibo dalle elevate proprietà salutistiche per la prevenzione di alcune patologie e in grado di garantire la sicurezza alimentare assume un ruolo chiave nello stimolare l'approfondimento della conoscenza da parte degli enti di ricerca e delle industrie agroalimentari degli aspetti qualitativi del prodotto ortofrutticolo utilizzato per il consumo fresco e trasformato o per la produzione di cibi arricchiti di composti nutraceutici.
Numerosi fattori influiscono sulla composizione e qualità del prodotto ortofrutticolo nelle fasi di pre- e post-raccolta, quali il genotipo (varietà e portinnesto), le tecniche agronomiche e la conservazione del prodotto. La possibilità di aumentare la biosintesi di alcuni componenti chimici, ed in particolare dei composti nutraceutici (flavonoidi quali i fenoli e le antocianine), rappresenta una importante strategia per l'ottenimento di cibi funzionali.
Scopo della tesi di Dottorato è la valutazione dei fattori agronomici e di post-raccolta che influenzano le caratteristiche qualitative e nutraceutiche dei frutti di arancia rossa e melograno. La ricerca è stata svolta sul prodotto frutticolo di queste specie che si distinguono per l'elevato contenuto di antocianine, carattere molto apprezzato dal consumatore attento alle proprietà salutistiche.
Lo studio sulle arance rosse ha riguardato diversi aspetti, quali l'influenza di alcuni portinnesti sulla precocità nell'entrata in produzione e sulla qualità del frutto, e l'effetto di alcuni trattamenti in post-raccolta sui parametri chimici e qualitativi del frutto.

Per quanto concerne il melograno la ricerca si è incentrata sull'evoluzione delle caratteristiche qualitative e nutraceutiche dei frutti nei seguenti studi: confronto di cultivar internazionali coltivate in due ambienti mediterranei, Spagna e Italia e caratterizzazione di numerose accessioni siciliane. Un'altra indagine ha riguardato l'analisi dell'espressione genica della biosintesi dell'antocianina durante la maturazione.
I risultati appaiono di notevole interesse per la comprensione del ruolo chiave che in ciascuno studio maggiormente influenza gli aspetti qualitativi e nutraceutici e per chiarire l'effetto dei singoli fattori agronomici e di post-raccolta.

List of abbreviations

AAPH	2,2'-azobis-(2-amidinopropane) dihydrochloride
AOC	Total antioxidant capacity
AUC	Area under curve
CCI	Citrus Colour Index
CIELAB	Commission Internationale de l'Eclairage
DPPH	1,1-diphenyl-2-picilhydrazyl equivalents
FAO	Food and agriculture organization
FRAP	Ferric-reducing capacity of plasma
GAE	Gallic acid equivalent
GC-MS	Gas chromatography-mass spectrometry
GMOs	Genetically Modified Organisms
HPLC	High Performance Liquid Chromatography
ORAC	Oxygen radical absorbance capacity
ROO	Peroxyl radical
ROS	Reactive oxygen species
TA	Titratable acid
TSS	Total soluble solids

INTRODUCTION

Quality concept in horticulture

1. *Definition*

'Quality' is the aptitude of a good (product) or service to satisfy the needs of its users.
ISO Standard 8402:1987 defines quality as "*The totality of features and characteristics of a product or service that bears its ability to satisfy stated or implied needs*".
For agrifood products, quality may be regarded as a complex characteristic of foods that determines its value and acceptability by consumers (22nd Regional FAO Conference for Europe, Oporto, 2000). The general concept of quality is complex and global because is a result of the biodiversity production and the inter-relations between links in the chain as safety, hygienic, nutritional and organoleptic characters; for the consumers, the concept of quality is extended on its use and service, as convenience (easy to use) and conservation.

2. The quality for fresh and processed fruits

Quality attributes for a product that fulfils needs and expectations of consumers (and other actors in the chain) belong to two main categories:
- product attributes, directly relating to the product attributes,
- process attributes, relating to production and processing.
The first include those relating to taste, appearance, texture, consistency, smell, safety and some functional characteristics, such as post-harvest life and convenience; the second, on the other hand, include among others, organic production, GMOs, environmental concerns and origin. Other quality attributes, such as microbiological and chemical contaminants or the nutritional value, are in

general not grasped through the consumer's experience or perception of the product and can only be conveyed by external indications, such as certifications or quality labels. Likewise are other process attributes, such as environmental impact, which can only be identified with attached labels or marks (http://www.fao.org/ag/agn/CDfruits_en/b_contenidos/3_packaging/txt_3/p1_activ1_3.html).

Many factors influencing composition and quality of fruits. The effects of pre harvest factors play a key role in order to obtain the optimum postharvest quality of vegetables, beginning very early in the farm planning process and improve during the harvest and storage processes. Generally, the quality concept can be defined in a broad sense as the grouping of inherent attributes perceived by the sense of taste (sweetness, acidity, bitter, astringency) and smell mostly related to internal quality while external standards are based on characteristics perceived by the sight and touch (texture, colour attractiveness or the presence of defects as blemishes or cracking), or other additional attributes with higher commercial, toxicological and nutritional implications (Tadeo et al., 2008). The fruit quality start in field, where it is possible generate and influence a lot of parameters and, then, the market destination.

The selection of cultivars and rootstocks are important factors to obtain fruit with quality that responds to processing and/or for fresh market sale. Pedoclimatic condition as temperature, light exposure, soil etc. have a strong influence on nutritional quality of fruits. For example, light intensity significantly affects vitamin concentration, temperature influences transpiration rate, which will affect mineral uptake and metabolism. Soil type

and/or nutrient and water supply affect significantly the mineral content of fruit; in vegetables, for example, nitrogen is involved in protein synthesis and its deficiencies in soil may lead to lower protein concentrations and the excess may reduce the vitamin C, while in fruit an excess may lower fruit sugar content and acidity (Silva, 2008).
At harvest time, the maturity stage of fruits is the most important parameter to consider as the primary factor that affect the physical-chemical composition and its storage life. Depending on the destination (for fresh or processed line), the optimal harvest time can change and fruits can be harvest or in its maturity stage, or very earlier in order to decrease mechanical damage during postharvest handling. About pre-harvest factors, the quality concept is directly influenced with abiotic (genetic) and biotic factors (climatic condition and cultural practices).

3. Main quality parameters and methodologies for their assessment

The appearance is considered the most important quality property that influence consumers to market and includes colour, shape, size and surface conditions.

3.1 Size

The changes in size of a crop as it is growing are used frequently as maturity criteria do to the size play an important role because is related to the market requirement and to determining the crop final price. For example, it is common to use in field templates to estimate the size of little fruits as sweet cherry and limes, or better an innovative digital caliper for medium fruits as tomatoes, citrus fruits,

banana, sweetcorn, or a ruler for big vegetables as pumpkin, watermelon, etc. (Fig. 1.1) (Thompson, 2003). The size depend on the equatorial diameter and polar height measurements.

Figure 1.1. Sizer used commonly in field (above left and right, respectively) and electronic digital slide gauge with 0.01 mm accuracy (below left and right, respectively)

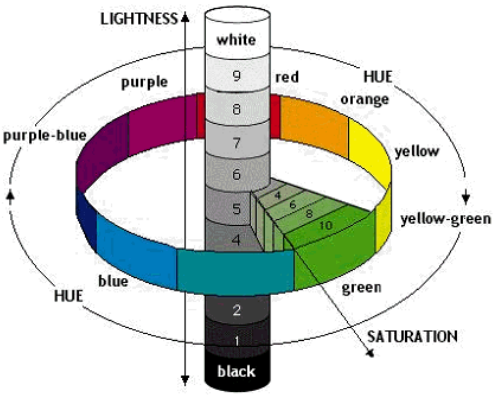
3.2 Colour

Colour is subject to perception. Different people interpret the expressions of colour in many different ways. For food, the colour parameter is commonly used as indicator of some

inner constituents because influence consumer's choice and preferences.
Colour measurement can be carried out in two main ways: visual evaluation with visual (sensorial) analysis and instrumentally using either colourimeters or spectrophotometers.
Because the spectral distribution of light is related to (source of radiant energy) the illuminant the object to which the colour is ascribed, the sensitivity of the eye of the observer is very different on colour perceived. The sensitivity of the eye varies even within this narrow visible range and is measurable in terms of intensity and wavelength; under moderate-to-strong illumination conditions, the eye is most sensitive to yellow-green light of about 550 nm. The instrumental measure is based on the absorption of a certain amount of radiation, basin of the Beer-Lambert's law for the spectrophotometers, while the instrumental colour system used to measure the colour space is the tristimulus colourimeter. Colourimeters give measurements that can be correlated with human eye-brain perception. Colour space transformation is the most common pixel pre-processing method for food quality evaluation. The CIELAB colour scales are the most popular space colour models used in food computer vision, because uniform the colour differences of an object in relation of human perception of differences; it is based on:
- L* (lightness) axis, where 0 is black and 100 is white;
- a* (red-green) axis, where the positive values are red and negative ones are green and zero is neutral;
- b* (blue-yellow), where the positive values are yellow and the negative ones are blue and zero is neutral;
- Chroma (C*), considered the quantitative attribute of colourfulness, is used to determine the degree of difference

of a hue in comparison to a grey colour with the same lightness and usually, the higher the chroma values, the higher is the colour intensity of samples perceived by humans;

- Hue angle (h*), considered the qualitative attribute of colour, is the attribute according to which colours have been traditionally defined as reddish, greenish, etc. and it is used to define the difference of a certain colour with reference to grey colour with the same lightness; this attribute is related to the differences in absorbance at different wavelengths and a higher hue angle represents a lesser yellow character in the assays (Fig. 1.2) (Pathare et al., 2012).



(Adapted from
http://www.regional.org.au/au/asssi/supersoil2004/s4/poster/1556_islamk.htm)

Figure 1.2. Munsell's cylindrical arrangement of colours. The horizontal line represents chroma (saturation), the vertical line represents value (lightness) and the circle represents hue

The different combinations of L*, a* and b* colour values have been fitted in different form of linear models, and are then used to predict food quality index as freshness, maturity stage, pigment evolution, defects or damages, etc (Jha, 2010).
In citrus contest, in 1981 Jiménez-Cuesta et al. have proposed a common model, namely 'Citrus Colour Index', in order to evaluate the degreening process on citrus fruit during maturation, using the formula:

$$\mathbf{CCI = (1{,}000 \times a^*)/(L^* \times b^*)}$$

In this preliminary study, Jimenez-Cuesta et al. (1981), according to the external colouration at the harvest time and the citrus variety considered, different ethylene treatments are recommended. In general, for orange fruits, it is assumed that the CCI should be >+7 for maturity fruits and the CCI requirements for degreening are between –5 and +3.
Nowadays in the citrus industry, peel colour is widely used as a commercial colour index in order to determine the correct harvesting date or to decide if citrus fruits should undergo a degreening treatment (DOGV, 2006).

3.3 Texture

Texture analysis is consider a parameter to testing cell structure and to determining shelf-life of food products. Physical testing of food products by texture analysis can tell us a lot about its tactile properties, such as firmness, fracture-ability, resilience, and others. More methods for measuring texture have been developed, and are categorized in subjective and objective one. The first is based on

sensorial analysis with a test panel, in order to affect the acceptance of a food product by consumer's sensory perception (appearance, tactile properties, aroma, flavour, off-flavour, taste) but results are quite variable and the test accuracy is not very good; the second one require a force gauge of some sort and are usually categorized into non-destructive and destructive methods. The non-destructive is based on compression, while the destructive on penetration and deformation. Applying a fixed force, expressed as kg/cm^2 or Newtons (N), the first cause the deformation to the surface of the fruit, while the penetration analysis, determined in peeled fruits using both the manual penetrometer with a cylinder probe of different mm of diameter, that the flagship texture analysis instrument equipped with probes or plates (Fig. 1.3).

(Adapted from http://www.foodtechcorp.com/tms-pro-texture-analyzer)

Figure 1.3. Example of flagship texture analysis instrument

For vegetables, and in particular for fruits, several factors affecting texture changing in pre and postharvest time. In preharvest, some elements are associate with texture quality as minerals, pedoclimatic condition, etc. For example, in tomatoes, are commonly use calcium treatment on foliar spray, in field, and/or in dip on fruits in order to increase cell wall calcium contents; for pear fruits, the liquid calcium fertilizer treatment on tree are a good option for the maintaining of texture and fruit weight loss during postharvest storage, but there was no effect on soluble solids contents (Moon et al. 2000). For strawberries, high calcium fertilizer levels reduced the acidity and played a part in loss of visual fruit quality after harvest (Lacroix and Carmentran, 2001).
The crops firmness change during maturation and especially during ripening stage, when texture rapidly may become softer. The temperature that subject crops during maturity can affect its ripening date, the overall quality and postharvest life.

3.4 Taste

The taste influence the overall food flavour, and depend by chemical components as organic acids and sugars and their content, and its balance change rapidly during the developmental stage. The 'flavor' is a sensory impression generated when food is consumed and is defined as an overall sensation caused by the interaction of chemical senses of taste and smell (odor) and textural feeling. The chemical senses are responsible of taste because depend by nonvolatile compounds at room temperature; this perception is correlated by taste receptors located in the taste buds of tongue, associating sweet, sour, bitter, salt and umami

(savory) taste. While salt and sugar are considered flavorants that enhance salty and sweet tastes, other secondary flavors are considered important and referred to as taste flavorants, namely 'umami' or 'savory' but commonly known as flavor enhancers, and composed by amino acids and nucleotides.

Carbohydrates are the most abundant organic compounds on earth for its central role in the metabolism of animals and plants. In plants, the biosynthesis start from carbon dioxide and water with light energy (i.e. photosynthesis). They act as sweeteners, gelling and stabilizers and are also precursors for aroma and colouring (in thermal processing like caramelization by Maillard reaction). All compounds composed by hydrates of carbon ($6C+6H_2O$) are identified as carbohydrates, and divided in mono, oligo and polysaccharides. Monosaccharides are polyhydroxy-aldehydes or ketones, generally with a unbranched C-chain, as glucose, fructose and galactose. Oligosaccharides are composed by <10 carbohydrate units for polymerization from monosaccharides with the elimination of water to give full acetals; the most representative are saccharose (sucrose), maltose, lactose, raffinose, etc. Polysaccharides consist of n monosaccharides, and the number n is >10; compared to mono and disaccharides, these polymers are less soluble in water and don't have a sweet taste because represent structural molecules, known as starch, cellulose and pectin (Belitz et al., 2009). During the ripening stage, generally there are the hydrolysis of starch in simple sugars, with an increase in content. Starch is broken down to sucrose by the action of sucrose phosphate synthetase and non-reducing sugars from sucrose by acid hydrolysis. Starch-sugar conversion is influenced by harvest maturity,

climatic condition, the stage in the respiratory of climacteric or non-climacteric fruit (Thompson, 2003).
Aroma substances are volatile compounds perceived by odor receptor sites in the olfactory tissue on the nasal cavity; in foods, there are known a total of 7100 compounds but only a limited number are important for aroma. Among these, exist a character impact aroma compounds called 'key odorants', responsible of the characteristic aroma of a food. The perception is correlated with the 'recognition threshold', i.e. the amount/concentration of volatile substances (expressed as mg kg^{-1}) determined by smelling (orthonasal value) and by tasting the sample (retronasal value) (Belitz et al., 2009).

4. Bioactive compounds and nutraceutical aspects

Crops are rich of nutrient and antioxidant compounds, and their quantity and quality are strongly influenced by variety, ripeness stage, pedoclimatic condition, and field. All of these can be divided in two group: the first is composed by sugars, polysaccharides, organic acids, N-compounds, lipids, minerals and vitamins and represent the nutritional part, while the second one include aroma and pigment as organoleptic and nutraceutical constituents. Many health-protective dietary phytonutrients found in crops are bitter, acid or astringent and therefore aversive for consumers, with a various removal during the industrial debittering processes or through selective breeding (Drewnowski and Gomez-Carneros, 2000). But the continuous interesting on healthy foods (e.g. functional foods) by consumers made a dilemma on its designing and acceptance.
In these few years, in food industry a lot of chemical substances are used as additive in order to maintain qualities

and characteristics that consumers demand, to prolong the shelf-life in postharvest, to keep food safe, wholesome and appealing from farm-to-fork. These non-essential substances are by 'chemical' or 'biological' origin and are widely known as 'bioactive compounds', that can be classified based on molecular identity or biopolymer type that includes polyphenolic compounds, indigestible carbohydrates (dietary fibers), functional lipids (mainly in cereals and seeds), proteins and peptides, vitamins and carotenoids. The health helpful effects depending on the dose and their availability, their absorption, metabolism, distribution, excretion and transport across cell membranes together with their ability to bind to specific receptors; in fact, some of these being beneficial at low levels of intake but harmful at higher exposure levels, and some ones might be benign for some sectors of the population and harmful for others (causing intolerance or allergenic reactions). These substances are categorized in two group:

- 'naturally-occurring', if are by biological origin and intrinsic components of the foods,
- 'chemical-occurring' or 'man-made', if their presence in food is due by addition.

Diary, many biological reactions are responsible to produce free radicals (reactive oxygen species) that damaging crucial biomolecules; for this reason, in this last years in medicine the most popular studies are based on the antioxidant role of many constituents, available in nature.

4.1 Reactive oxygen species (ROS)

ROS are chemical compounds which have a tendency to donate oxygen to other substances, with destructive actions on both DNA and proteins; they are continuously produced

as byproducts of aerobic metabolism and, depending on the nature of the ROS species, some are highly toxic and rapidly detoxified by various cellular enzymatic and non-enzymatic mechanisms (as vitamins and phenolic compounds). ROS as generated also for abiotic (UV radiation, light, environmental pollution, food dietary) and biotic stress conditions including pathogen defense, programmed cell death (apoptosis), all implicated in playing an important role in chronic degenerative disease, as cancer, inflammatory, cardiovascular and neurodegenerative diseases, and ageing (Simon et al., 2000).

Atoms or groups of atoms as hydroxyl radical (•OH), superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), oxygen singlet ($^1O_2$), hypochlorite (ClO^-), nitric oxide radical (•NO), lipid peroxide radicals (ROO•) and peroxynitrite radical (NO_3^-) are common namely 'free radicals', because contains an unpaired electron in an atomic orbital (sometimes unstable and highly reactive), and are able either to donate or to accept an electron from other molecules, therefore behaving as oxidants or reductant. The harmful actions of free radicals can be blocked by scavenging of antioxidant compounds.

There is a huge number of phytochemicals with antioxidant properties in plant-based foods, and among bioactive compounds the most important known are alkaloids, glucosinolates, terpenes, polyphenols, vitamins, carotenoids. The antioxidant protection of these compounds increase if derived from the diet, and when are absorbed and made systemically available, it is observed an important improvement of their endogenous defense.

4.2 Alkaloids

Are classify as basic nitrogenous compounds in which the nitrogen is usually contained within a heterocyclic ring system, has protective function in plants and a pharmacological action; the most known are caffeine, theobromine, morphine, solanine, nicotine, piperin, adrenalin, noradrenalin and serotonin.

4.3 Glucosinolates

Are amino acid-derived secondary plant metabolites found exclusively in cruciferous plants and *Brassica* species, and their breakdown products (sinigrin, progoitrin, glucobrassicin) shown nutritive and antinutritional properties, potential adverse effects on health, anticarcinogenic properties and characteristic flavour and odour of *Brassica* vegetables; 120 different glucosinolates are characterized and the levels may depend on variety, cultivation conditions, climate and agronomic practice, and in vary parts of the plant and their classification of glucosinolates depends on the amino acid from which they are derived (i.e. aliphatic glucosinolates derived from alanine, leucine, isoleucine, methionine or valine; aromatic glucosinolates derived from phenylalanine or tyrosine and indole glucosinolates are derived from tryptophane (Sørensen, 1990).

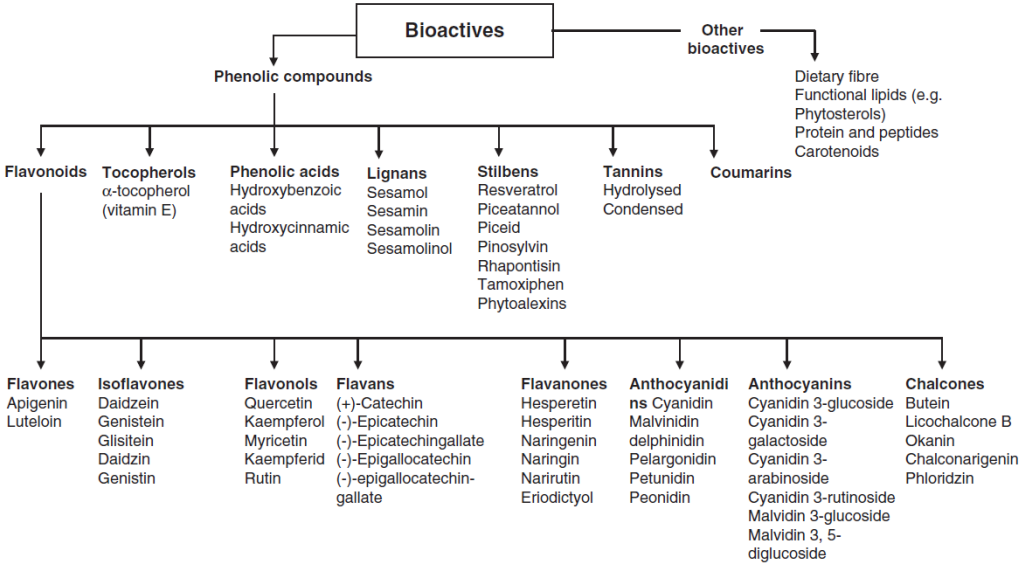
4.4 Terpenes

Group of molecules whose structure is based on a number of isoprene units as methylbuta-1,3-diene named ‘hemiterpene’ with 5 carbon atoms and play protective and aroma function

in plants (i.e. eugenol, cinnamon, limonene, camphor, eugenol, geraniol, menthol, etc.) (Gilbert and Şenyuva, 2008).

4.5 Phenolic compounds

Occur as plant secondary metabolites, are widely distributed in the plant kingdom and in human diet a corresponding antioxidant capacities with health benefits, and chemically are defined by the presence of at least one aromatic ring bearing one (phenol) or more (polyphenols) hydroxyl substituents, including their functional derivative (e.g. esters and glycosides). Several classes can be considered according to the number of phenol rings and to the structural elements that bind these rings. About polyphenols, we differ two main groups: flavonoids and non-flavonoids. (Fig. 1.4).

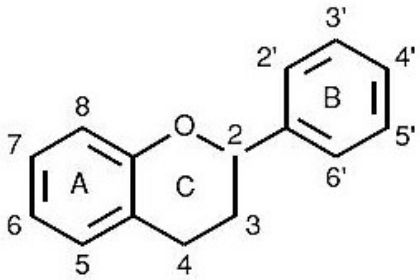


(adapted from Skinner and Hunter, 2013)

Figure 1.4. Possible classification and examples of plant bioactive compounds.

4.5.1 Flavonoids group

Are characterized by a basic structure C6-C3-C6 (skeleton of diphenylpropane), as a two aromatic benzene rings (ring A and B) linked by a three-carbon aliphatic chain which is condensed to form a pyran or a furan ring (heterocyclic ring containing oxygen namely ring C) (Fig. 1.5).



(adapted from Skinner and Hunter, 2013)

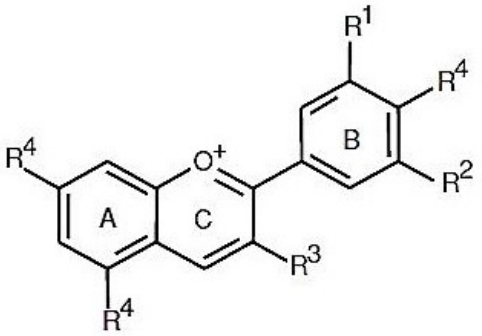
Figure 1.5. Chemical structure of flavonoid 'backbone'.

Two different subgroups classify flavonoid depending on the carbon of the C ring on which B ring is attached, and the degree of unsaturation and oxidation of the C ring; the first subgroup is namely **isoflavones,** in which B ring is linked in position 3 of the ring C, while the second one **neoflavonoids**, those in which B ring is linked in position 4. Flavonoid in which the B ring is linked in position 2 can be further subdivided into several subgroups on the basis of the structural features of the C ring.

Flavones, characterized by the presence of a double bond between positions 2 and 3 and a ketone in position 4 of the C ring.

Flavonols, characterized by a hydroxyl group in position 3 of the C ring, which may also be glycosylated.
Flavanones, namely also dihydroflavones, differ structurally for the absence of the double bond between positions 2 and 3, because the C ring is saturated.
Flavanonols, namely also dihydroflavonols, are the 3-hydroxy derivatives of flavanones; they are an highly diversified and multisubstituted subgroup.
Flavanols (or flavan-3-ols or catechins) present the hydroxyl group almost always bound to position 3 of C ring, and there is no double bond between positions 2 and 3 and the lack of a carbonyl group, that is a keto group in position 4; for these reasons, flavanols have two chiral centers in the molecule, on positions 2 and 3, then four possible diastereoisomers. The isomers are distinguished for the configuration in 'epicatechin', if is cis and 'catechin' if is trans; each of these configurations has two stereoisomers, namely, (+)-epicatechin and (-)-epicatechin, (+)-catechin and (-)-catechin.
Then, flavanols have the ability to form polymers, called 'proanthocyanidins' or 'condensed tannins', because of an acid-catalyzed cleavage produces anthocyanidins.
Anthocyanidins, chemically known as flavylium cations, are generally present as chloride salts and the sugar are free molecules. All flavonoids are colourless, while only anthocyanidins gives plants colours depending on some factors as the pH and the methylation or acylation at the hydroxyl groups on the A and B rings.
Anthocyanins are glycosides of anthocyanidins. Sugar units are bound mostly to position 3 of the C ring and they are often conjugated with phenolic acids, such as ferulic acid, Depending on the number and position of hydroxyl and

methoxyl groups, various anthocyanidins have been described and of these, six are commonly found in vegetables and fruits: pelargonidin, cyanidin, delphinidin, petunidin, peonidin, malvidin (Fig. 1.6).



(adapted from Skinner and Hunter, 2013)

Figure 1.6. Chemical structure of anthocyanin molecule.

Sugars are linked mainly to the C3 position as 3-monoglycosides, to the C3 and C5 positions as diglycosides (with the possible forms: 3-diglycosides, 3,5-diglycosides, and 3-diglycoside-5-monoglycosides). Glycosylations have been also found at C7, C3 and C5 positions; when there are several acylated sugars in the molecule, these anthocyanins are sometimes called 'polyglycosides'.
To the sugar unit of different acyl substituents such as:
- aliphatic acids, such as acetic, malic, succinic and malonic acid;
- cinnamic acids (aromatic substituents), such as sinapic, ferulic and p-coumaric acid;
- pigments with both aromatic and aliphatic substituents.

4.5.2 Non-flavonoids group

This group is classified according to the number of carbons that they have and comprises in subgroups.

Simple phenols (C6) are formed with an aromatic ring substituted by an alcohol in one or more positions as they may have some substituent groups, such as alcoholic chains, in their structure.

Phenolic acids (C6-C1) are simple phenols with a carboxyl group linked to benzene.

Hydrolyzable tannins are mainly glucose esters of gallic acid, and the most known are two types: the gallotannins, which yield only **gallic acid** upon hydrolysis, and the **ellagitannin**s, which produce ellagic acid as the common degradation product.

Chalcones and **dihydrochalcones** are flavonoids with open structure and are classified as flavonoids for the similar synthetic pathways. They are water soluble pigments and are present in the vacuolar sap of the epidermal tissues of flowers and fruit.

Hydroxycinnamic acids, included in the phenylpropanoid group (C6-C3), are structured by an aromatic ring and a three-carbon chain; the most widespread are the coumaric, caffeic, ferulic and sinapic acids, but in nature they are usually associated with other compounds such as chlorogenic acid (which is the link between caffeic acid and quinic acid).

Acetophenones and **phenylacetic acids** both have a C6-C2 structure, and are aromatic ketones the first and have a chain of acetic acid linked to benzene, the second one.

Benzophenones and **xanthones** present the C6-C1-C6 structure. The basic structure of benzophenone is a diphenyl ketone, and that of xanthone is a 10-oxy-10H-9-oxaanthracene.

Stilbenes have a 1,2-diphenylethylene as their basic structure (C6-C2-C6) and in plants are present as cis and trans isomers (obtained by UV radiation). Resveratrol, the most widely known compound and contains three hydroxyl groups in the basic structure.

Lignans are compounds derived from two β-β'-linked phenylpropanoid (C6-C3) units and are widely distributed in the plant kingdom. They are classified into eight subgroups, based upon the way in which oxygen is incorporated into the skeleton and the cyclization pattern: furofuran, furan, dibenzylbutane, dibenzylbutyrolactone, aryltetralin, arylnaphthalene, dibenzocyclooctadiene, and dibenzylbutyrolactol.

Secoiridoids are complex phenols produced from the secondary metabolism of terpenes as precursors of several indole alkaloids, and characterized by the presence of elenolic acid, in its glucosidic or aglyconic form, in their molecular structure. An exemple of secoiridoids is the oleuropein, responsible of the typical bitter and pungent taste of *Olea europea* fruits and chemically is a heterosidic ester of elenolic acid and 3,4-dihydroxyphenylethanol containing a molecule of glucose, the hydrolysis of which yields elenolic acid and hydroxytyrosol (De la Rosa et al., 2010).

4.6 Vitamins

Vitamins are organic compounds consider essential for the renowned good biological activity in human diet. They are divided in two classes, based on their solubility and ability to dissolve into another substance, in fat-soluble and water-soluble.

4.6.1 Fat-soluble vitamins

This group include A, D, E and K1 vitamins. This particular structure allow it to be stored in human fat, because are located in lipid-rich structures such as cell membranes and lipoproteins, protected from oxidation by the polyunsaturated fatty acids.

Vitamin A in human is present in three active forms, namely respectively retinol, retinal, and retinoic acid; main physiological effect of carotenoids in humans has been classically attributed to their role as provitamin A, since those carotenes with a β-ring end group are converted to vitamin A (retinol) by the action of an intestinal monooxygenase (i.e. the most notably is the β-carotene).

With the general name of **Vitamin E** it speak about a group of eight lipophilic compound, i.e. α-, β-, δ- and γ-tocopherol and α-, β-, δ- and γ- tocotrienol, that differ in the number and position of the methyl groups on the ring. The main sources are vegetable oils, in particular germ oils of cereals. In humans, vitamin E is the major lipid-soluble chain-breaking antioxidant, found in all cell membranes and plasma lipoproteins because plays an important role in the biosynthesis of haemoglobin, and it is renowned to help to

protect low density lipoproteins, nucleic acids, and polyunsaturated fatty acids from oxidative damage.

4.6.2 Water-soluble vitamins

This group include B1, B2, B6, nicotinamide, pantothenic acid, biotin, folic acid, B12 and C vitamins (Gilbert and Şenyuva, 2008). The main ROS scavenging antioxidants in vegetables and fruits are **vitamin C** (ascorbic acid), a colourless water soluble compound that have an important role and in human biology because is a cofactor in the synthesis of collagen and prevent the scurvy diseases; in fruits, the content of vitamin C depend by several factor as type of cultivar, growing conditions and stage of ripeness, and it is very labile because can be destroyed by heat, light and exposure to air.

Sweet orange (*Citrus sinensis* L.)

1. Taxonomy and origin

On the history, different hypotheses have been formulated about the geographical origin and the plantation area of citrus; until the mid of 1900s, Citrus taxonomic systems were based on morphological and anatomical differences and on the geographical area of origin. Morphological traits about tree, floral biology, farming and different uses and properties of fruits were described a long time ago by Theophrastus (*Historia plantarum*, 313 AD), Virgil (Georgics, 30 AD), Dioscorides (*De materia medica*, 60-79

AD), Pliny the Elder (*Naturalis historia*, 27-79 BD), and so on.

A very important citrus taxonomy contribution was first done in 1646 by John Baptista Ferrarius, a Jesuit priest and botanist of Siena, in his book *Hesperides, sive de malorum aureorum cultura et usu*, and after enhanced thanks his relationship with Cassiano dal Pozzo, who provided several drawings, done in tempera, of life-size fruits, today preserved in the Royal Library of Windsor and in some private collections.

A few years later, a large number of authors described citrus fruits in less detail (Steerbeck, 1682; Hermann, 1687; Tournefort, 1700), but a complete change of the classification system of citrus is given by Linneus (1737) who, in his work *Genera plantarum*, created the genus 'Citrus', attributing three main species to it:

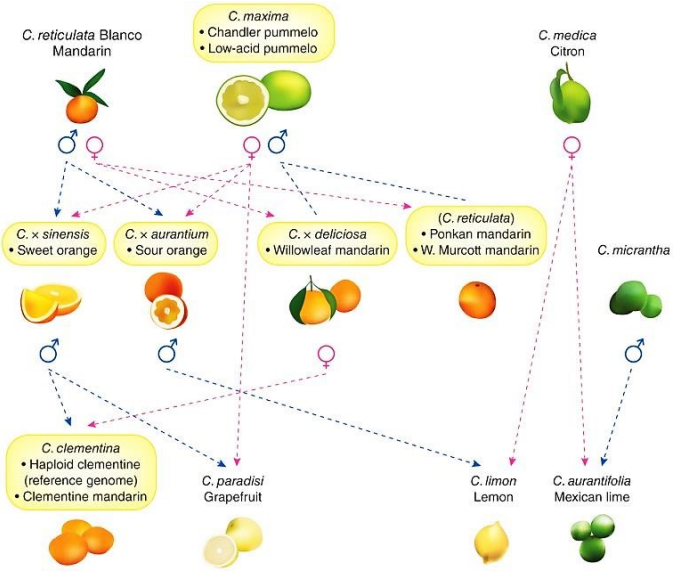
- *Citrus medica* (citrons and lemons),
- *Citrus aurantium* (sweet and sour oranges and the pummelo),
- *Citrus trifoliata*,

and next a collaboration with Osbeck, were formulated the binomial names of three species: *Citrus grandis*, *Citrus limonia* and *Citrus sinensis.*

The first citrus paper was published by Giorgio Gallesio (1811) in the *Traité du Citrus* in Paris, with an important contribution to innovative citrus taxonomy and describing citrons, lemons, sour oranges and their hybrids accompanied by citrografic atlas containing colour table of the main varieties of citrus. Then, Citrus taxonomic systems of Hooker (1875) and Engler (1896) were based on morphological and geographical data, proponing the first 13, and the second 11, genera of the Aurantioideae.

The 1943 was an important year in which was published the work of W.T. Swingle, *The botany of Citrus and its wild relatives of the orange subfamily*, where was accepted the Engler classification and dividing the genus *Citrus* in two subgenera, *Citrus* and *Papeda*, which included, respectively, ten and six species, and separated according to their morphological characteristics and to the biochemical composition of different parts of the citrus plant as flowers, leaves and fruits. A very similar but much more complex was the contemporary Tanaka's taxonomy works (1954; 1961), *Revisio aurantiacearum* and *Species problem in Citrus*, where the genus *Citrus* was divided in two subgenera, *Archicitrus* and *Metacitrus*, 8 sections, 15 subsections, 9 groups, 2 subgroups, 2 microgroups and 157 species. The big difference in number of species recognized in these two systems and some intermediate ones reflected opposing theories on what degree of morphological difference justified species status. In order to heal the rift between these theories, in 1961 Hodgson proposed a new classification, increasing to 36 the number of species and dividing them into four groups: 'acids fruits', 'orange group', 'mandarins group' and 'other'.

But there is definitely no single method to classify *Citrus*, and already in 1976 Barrett and Rhodes and recently Wu G.A. et al., (2014) have suggested to consider only three citrus types as 'valid' or 'true' species, namely citron (*Citrus medica*), mandarin (*Citrus reticulata*) and pummelo (*Citrus grandis*, now called *C. maxima* Burm. Merrill), indicating the ones as the progenitors of citrus and some *Citrus* and related genera (Fig. 2.1).



(adapted from Velasco and Licciardello, 2014)

Figure 2.1. The origin and evolution of select citrus species

Thanks to the modern biochemical and molecular techniques, this latter classification was confirmed in particular with DNA markers, but the Swingle' classification is the most widely used (Khan, 2007).

The C*itrus* germoplasm and its related genera is very large, and the general origin area is believed to be in the tropical and subtropical regions of South-east Asia – north-eastern, India, southern China, the Indo-Chinese peninsula – and the Malay Archipelago, and then spread to other continents (Webber, 1967; Chapot, 1975), but a recent evidence (Tolkowsky, 1938) suggests that the mountainous regions of

southern China and north-east India may be the center of origin.

For oranges, there are two species: sour orange (*C.aurantium* L.), used as rootstock and sweet orange [*C.sinensis* (L.) Osbeck]. Worldwide, during history the major species of *Citrus* have occurred extensive movements and nowadays it are separated in five economically important species:

1. sweet oranges [*C.sinensis* (L.) Osbeck],
2. mandarins (*C.reticulata* Blanco and *C.unshiu* Marc.),
3. grapefruits (C.*paradisi* Macfadyen),
4. lemons [*C.limon* (L.) Burmann *f.*],
5. limes (*C.aurantifolia* Christm. Swingle).

As regards the sweet orange [*C.sinensis* (L.) Osbeck], that is considered as the most widely commercialized among the citrus species, several researchers confirm full agreement on its hybrid origin (*Citrus maxima* Burm. Merrill X *Citrus reticulata* Blanco); although the presence of a lot of varieties originated by mutation, sweet oranges are thought to be hybrids (Barrett and Rhodes, 1976; Torres et al., 1978; Scora, 1988; Fang and Roose, 1997; Nicolosi et al., 2000).

2. Economic importance and world diffusion

Due to economic developments and the people lifestyle change, fresh consume is increase in particular for fruits of category 'easy-peeling' and 'seedless' as tangerine/mandarins and oranges; citrus is the most widely worldwide produce for its economic importance in 186 countries and world citrus production increased more (4.5%) every year during 1900s (Ladaniya, 2008), with other 133 million tones mark (FAOSTAT, 2011-2013).

Almost half of worldwide production is distributed in the Americas (North and South), followed by China (other 31%) and Mediterranean areas (23%), where Spain is the first citrus fruit producer (about 6 million tons) followed by Italy (3 million tons). About orange fruits production, Brazil leads in citrus worldwide production (18 million tons) and the Spain for the European countries (3 million tons), followed by Italy with about 2 million tons (FAOSTAT, 2011-2013), reaching as the 8th producer.
In Italy the cultivation areas are concentrated mainly in the Southern Italy, particularly in Sicily (50%), characterized to a long standing tradition in citrus growing. In Sicily, the citrus cultivation is very variegated and distributed; about oranges group, the 70% is constituted of the pigmented ones - 'Tarocco', 'Moro', 'Sanguinello' and 'Sanguigno' (has almost disappeared) varieties – only concentrated in the foothills of the Etna volcano, and the 30% of blond ones (Tribulato and Inglese, 2012). Along the oriental coast there is concentrate the lemon industry with 'Femminello' (95%), 'Monachello' (2%) and 'Interdonato' (3%) cultivars (Pergola M. et al., 2013). Over the last few decades, Italian citrus fruit producers have been losing their competitive edge to both the foreign and domestic markets (Baldi, 2011).

3. Morphological and physiological aspects

Citrus trees are evergreen shrubs, grown best in frost-free regions and exhibit a long juvenility (two to five years until first flowering), generally inversely related to tree vigour and heat unit accumulation. The seeds are exalbuminous with a coat surrounding a much reduced nucellus and

endosperm, and contains two cotyledons and from one to as many as seven embryos; only one of this is derived from the sexual fusion of the sperm and egg cells, while the additional embryos originating from nucellar tissue which is genetically the same as the diploid maternal tissue. For lemon and limes, this period is around two years under subtropical growing condition, while of 5 to 13 years may occur for mandarins, sweet oranges grapefruit when grown from seeds. Duration of juvenility is hardly affected by temperature, moisture and particularly by edaphic conditions.

When trees are mature, and when the winter temperatures decrease, buds start the induction developing the capacity to flower, then the differentiation (evocation) period which precede anthesis (flowering). During flowering arise five basic types of growth:

1. generative shoots (leafless or bouquet bloom), with flowers only borne on previous season's growth,
2. mixed shoots with a few flowers and leaves,
3. mixed shoots with several flowers and a few large leaves,
4. mixed shoots with a few flowers and many leaves,
5. vegetative shoots with only leaves.

All of the mixed shoots produce flowers and leaves in the new growth flush (leafy blooms) and the abundance depending on winter temperature (Davies and Albrigo, 1994).

Generally, it have start the leafless inflorescences, containing a bouquet of flowers with low probability to set fruit. On the other hand, flowers in leafy inflorescences that can be terminal or distributed among leaves along the shoot are commonly associated with higher fruit set (Jahn, 1973).

Citrus species usually produce a large number of flowers over the year. Sweet oranges [*C.sinensis* (L.) Osbeck], for example, may develop 250,000 flowers per tree in a bloom season although only a small amount of these flowers (usually less than 1 %) becomes mature fruit (Erickson and Brannaman, 1960; Goldschmidt and Monselise, 1977).

Various physiological traits difference cultivar; sweet oranges, mandarins, lemons and grapefruits show some degree of apomixis and/or parthenocarpy, sterile or self-incompatible and/or develop defective pollen (Baldwin, 1993; Davies and Albrigo, 1994). But for seeded citrus cultivars, fruit development depends upon pollination and fertilization with fruits rich of seeds. If the flower is not pollinated, the development of the gynoecium arrests, the whole flower senesces and eventually abscises (Davies and Albrigo, 1994).

Citrus cultivars, namely 'seedless varieties', show high parthenocarpy in many instances due to gametic sterility. Generative sterility can be relative or absolute. The absolute gametic sterility is associated with pollen and/or embryo-sac sterility, while relative gametic sterility may be due to self-incompatibility (as in Clementine) and to cross-incompatibility. Some cultivars such as Washington Navel oranges and Satsuma mandarins have both, although even in these two varieties a few embryo sacs may often reach maturation. In these varieties, parthenocarpic fruit are 'seedless' and therefore all pollination, fertilization or seed requirements for fruit growth activation have clearly been substituted by endogenous signals. Self-incompatible cultivars show a low degree of parthenocarpy and therefore can be considered to possess "facultative parthenocarpy"

meaning that seedless fruit form only when fertilization does not occur.

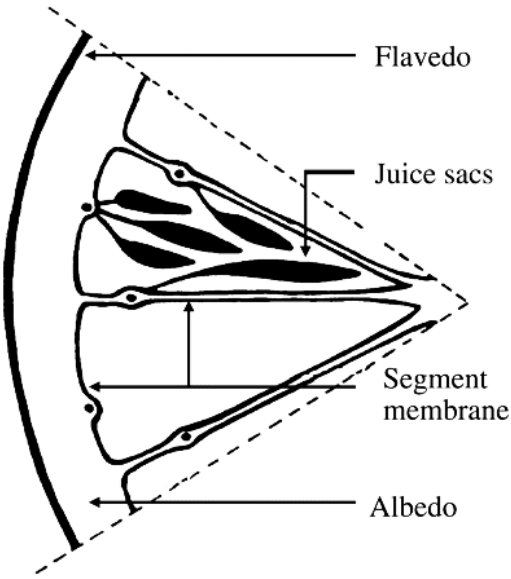
In the initial drop period occurs - from flowering until 3-4 weeks post bloom - the abscission of 'weak' flowers and fruitlets with defective styles or ovaries, or flowers which did not receive sufficient pollination, while 6-8 weeks after bloom most fruit abscise at the zone between the pedicel and the stem. In this period it may occur a disorder namely 'physiological drop' with the abscission of fruitlets of 0.5-2.0 cm in diameter, generally due to an important competition for carbohydrates, water, hormones and other metabolites or temperature stress and water deficit (Iglesias et al., 2007).

Citrus fruits are big berries namely 'hesperidiums' and during growth follow 4 sigmoidal phases where occur biochemical and physiological changes:

- phase I: cell division, where are produce all the cells of the mature fruit,
- phase II: cell differentiate, into various tissue types such as juice sacs, albedo, flavedo, etc.,
- phase III: cell enlargement, with a rapid increase of fruit size and % of sugar (TSS) and peel colour degreening,
- phase IV: maturation, with a decrease of total acidity (TA) and TSS:TA ratio balancing, peel colour and size conformity (Davies and Albrigo, 1994).

The fruit is composed of two major, morphologically distinct regions: the pericarp (peel or rind) and the endocarp (pulp), as the edible portion. The pericarp is further divided into two parts: the exocarp (flavedo), which is the external coloured portion and the mesocarp (albedo), the white layer of the peel. The pulp consists of segments, the ovarian locules, enclosed in a locular membrane and filled with the

juice vesicles that are the ultimate sink organ of the citrus tree (Fig. 2.2) (Iglesias et al., 2007).



(adapted from Liu et al., 2007)

Figure 2.2. Diagrammatic cross-section through a citrus fruit

Based on the chemical-morphological characteristics and for convenience of fruits, the sweet oranges may be divided into four groups, respectively named as 'the common or round', 'navel', 'pigmented or blood' and acidless ones. The common oranges, also known as blonde orange, is the most widespread in the world (Davies and Albrigo, 1994).

The first reference about a pigmented orange appears in the Hesperides of Ferrari (1646), in which he describes about an '*Aurantium indicum with a blood flesh*', introducing in Italy through a missionary from Genova returning from Filippine.

4. Factors affecting fruit quality

4.1 Cultivar

In the Middle Age, in the Mediterranean basin, the sour orange was the first introduced and cultivated. Was only around the mid-15th-century that Portuguese introduced the sweet orange from China, followed by its rapid development thanks to particular climatic condition present this area. Today the commercial production of sweet orange is based among four group: common or blond orange, navel group and blood ones. Citrus trees tend to produce spontaneous mutations very readily (i.e. in mandarin as Clementine varieties), in particular in nucellar seedling. Navel oranges and grapefruit produce more mutations than other citrus, but now there are an increasing of artificial induction using ionizing radiation (i.e. to generate pigmentation in grapefruit varieties, 'Star Ruby' and 'Rio Red').
How you can see above, sweet oranges are classified into four group: 'the common or round', 'navel', 'pigmented or blood' and 'acidless' ones.

Common orange
In this group are present blond or white varieties with potential importance for both processing and for fresh consuming. The commercial calendar start in November and ends in May. The most important worldwide varieties are:

‘Hamlin’ and ‘Pineapple’ in Florida, ‘Jincheng’ in China, ‘Shamouti’ in Israel, ‘Pera’ in Brazil, ‘Ovale’ or ‘Calabrese’, ‘Belladonna’ and ‘Common blond’ in Italy, ‘Valencia Late’, ‘Cadenera’, ‘Berna’ and ‘Salustiana’ in Spain, ‘Midknight’ in Mediterranean areas (Saunt, 2000).

Navel orange

This group represent all primary fruits having a distinctive small secondary fruit embedded in the apex, namely ‘navel’. This characteristic is sometimes found in oranges and particularly in mandarin and depend upon climatic condition, although are varieties at earliest maturity. Genetically, navels are very unstable and for this reason the variety selection have been made by growers in the past, obtaining fruits easy peeling, seedless and with large size.

The original variety is ‘Washington navel’, by a bud mutation of the portuguese ‘Selecta’, but there are most known varieties deriving to mutations, namely respectively ‘Navelina’ and ‘Newhall’ (originating from California and most widespread in Spain and Italy), ‘Navelate’ (originating and widespread in Spain), ‘Cara Cara’ (originating from Venezuela and distinguishably by deep red flesh pigmentation), ‘Lane Late’(originating from Australia) (Saunt, 2000).

Pigmented orange

‘Pigmented’ or ‘blood’ oranges are common orange characterized by red pigments (anthocyans) in the flesh and juice and sometimes in the rind. The origin is associate in Mediterranean area, probably in Sicily, where the best quality of orange supply is represented by the production of these ones, having some special flavor and organoleptic

characteristics that cannot be found outside Sicily, and in particular outside the east side of Sicily and in the south and south-west of Mount Etna. Red orange growing in Sicily is extremely important in some areas which are specifically suitable for their pedological and weather conditions. Owing to temperature ranges between morning and night, sometimes of beyond 20°C, there is an important contribute to the synthesis of anthocyanins. The characteristics of the land and climate are essential for producing the pigments that give red oranges their characteristic colour in some Sicilian territories. The essential factor is, indeed, whether the above-mentioned sudden change in temperature occurs when oranges ripen. This phenomenon, a characteristic of the Mediterranean, does not exist in tropical areas from which citrus fruits come. The interest in red oranges among consumers is due to different factors, including good taste and higher biological properties with respect to blonde oranges determined by the presence of anthocyanins. There are a lot of varieties commercialized on Mediterranean areas, namely Tarocco, Moro and Sanguinello (Italian origin) (Fig. 2.3), Doble Fina and Sanguinelli (Spanish origin), Maltaise sanguigne (Marocco origin) (Saunt J., 2000).

Figure 2.3. Fruits of Moro, Tarocco and Sanguinello cultivar (from left to right)

Among blood oranges, in Italy, Tarocco is the most known and widespread (58%, against 20% and 22% of Moro and Sanguinello, respectively). It seems that it could be a mutation of Sanguinello and its origins are in the last century in Francofonte (Sr) (Zarbà and Pulvirenti, 2006).
The most important characteristics are the good fruit size and the easy peeling character. During the last 40 years, different selections were isolated and characterized. Nucellar or micrografted selections were used to ensure free virus propagation material (Reforgiato Recupero and Tribulato, 2000; Reforgiato Recupero and Russo, 2001, 2002).

Others

Over the last few years, the worldwide citrus market require easy-peeling and seedless citrus fruit, in particular for mandarin and mandarin-like ones. In many breeding programs, the aim of research is to isolate diploid possessing good characteristics.
Researchers of CREA-ACM of Acireale (Reforgiato Recupero et al., 2005) have patended some hybrids and the most interesting is a triploid mandarin-like namely '**Mandared**', obtained from a cross between the Clementine Oroval (female parent, 2x) and Tarocco tetraploid (male parent, 4x). The age of maturation is medium-late (February-March) and fruits present a thin and easy-peeling skin, juicy and intense pigmented pulp, with a balanced acid-sugar ratio, consider a value added for its healthy effects. The fruit size is intermediate between that of orange and clementine.

5. Rootstock

Because of the long juvenility period and important susceptibility to several soil-related problems (*Phytophthora parasitica* and nematodes) of seedling trees, most citrus orchards worldwide consist of two-part (namely 'trees budded') trees that combine favorable attributes of the scion and rootstocks. For this reason, the selection of a good rootstock it's fundamental for tree performance and fruit quality.

Several researches in citrus-producing countries such as California, China, Spain has been and are currently done in order to evaluate CTV-tolerant rootstocks for the replacement/reconvertion of citrus orchards (Louzada et al., 2013; Caste, 2010; Intrigliolo and Reforgiato Recupero, 2011).

Nutritional and nutraceutical quality of oranges such as polyphenols (Rapisarda et al., 1999; Grosso et al. 2013) and anthocyanins content (Maccarrone et al., 1983, 1998; Rapisarda et al., 2000; Hyoung, 2000; Rapisarda and Russo, 2000; Dugo et al., 2003; Proteggente et al., 2003), in particular for Tarocco's clones (Rapisarda et al., 2000; Pallontino et al., 2012) grafted in sour orange have been extensively investigated, but no one studied it on new rootstocks yet.

Sour orange

For a long time, sour orange (*Citrus aurantium* L.) was considered the best rootstock for citrus, commonly used on poorly or heavy soil, due to its resistance to many fungal disease such as *Phytophthora* spp. - causing foot rot - and

tolerance to viroids such as exocortis (CEV) and xyloporosys - causing tree stunting, bark sloughing or stem pitting (Davies and Albrigo, 1994). The fruit quality of oranges and mandarins cultivated on sour orange is considered excellent: medium-large size, high content of total soluble solids (TSS) and low levels of titratable acidity (TA). However, due to its susceptibility to the *Citrus tristeza virus* (CTV) and the spread of this disease in Italy, the research on the whole range of graft-compatible Citrus species and Citrus relatives tolerant to CTV has recently been the main activity in order to obtain pathogen-tolerant citrus rootstocks (Mennone and Catalano, 2014).

Citranges

Citrange rootstocks, an intergeneric hybrids of sweet orange (*Citrus sinensis*) X trifoliate orange [*Poncirus trifoliata* (L.) Raf)], have found wide acceptance in recent years because exhibits tolerance or resistance to pests and diseases like CTV, cold and calcareous soils (Gmitter et al., 1996). This group was made in Florida beginning in 1897 and several rootstocks were tested, including 'Rusk', 'Morton', 'Savage', 'Benton', 'C-35', 'Carrizo' and 'Troyer'; the last two actually arose from the same cross between 'Washington' navel orange (seed parent) and *P.trifoliata* (pollen parent) made in 1909. About all, actually the most commercialized are:

- Troyer, originated as a hybrid of *C.sinensis* (L.) Osbeck cv. 'Washington navel' sweet orange X *P.trifoliata* (L.) Raf., was made by Savage under the direction of Swingle of the U.S. Department of Agriculture, at Riverside, California, in 1909. It is the more tolerant for Psorosis (CPsV), Citrus cachexia viroid (CCaVd) and *Phytophthora* spp. root rot,

cold, calcareous solis (active calcareous max 13,37%) and *Radopholus similis* nematode, but do not Exocortite (CEVd);

- Carrizo, that has been assumed as sister seedlings of Troyer (*C.sinensis* (L.) Osbeck cv. 'Washington' sweet orange X *P.trifoliata* (L.) Raf.), was made into the Winter Haven substation (No. 19) near Carrizo Springs, Texas. Troyer and Carrizo are indistinguishable because resulted from the same series of pollinations, but Carrizo, in contrast to Troyer, is considered burrowing nematode resistant and sensibility for *Fusarium* spp. (Savage and Gardner, 1965);

- C 35 (*C.sinensis* (L.) Osbeck cv. 'Ruby' x *P.trifoliata* (L.) Raf.), it is resistant to the citrus nematode (Tylenchulus semipenetrans Cob.). and trees on this rootstock also reach a smaller size than on Troyer or Carrizo citranges (Cameron and Soost, 1986) and the highter sensibility on ferric chlorosis (Forner-Giner et al.,2003).

The original reason for developing citranges was to produce fruits more freeze-hardly than sweet orange ones, but scion cultivars budded on it produce vigorous trees. 'Carrizo' and 'Troyer' are planted as rootstocks for oranges and grapefruit for their easily propagation and because produce seedy fruits with high incidence of nucellar embryony (Davies and Albrigo, 1994). Tarocco' clones on citrange rootstocks produce good crop of fruit: thin skin, hight texture, higher solid soluble content and strong red anthocyanic pigmentation.

Citrumelos

Citrumelos are intergeneric hybrids of grapefruit (*C. paradisi* Macfadyen) X trifoliate orange [*Poncirus trifoliata* (L.) Raf)] and the original crosses were made in 1907 in

Florida by Swingle and the most widely propagated as namely 'Swingle'.

- Swingle, identified as CPB 4475, in 1974 was called "ultra-resistant" by Wutsher because its several qualities such as biotic (CTV, *Phytophthora* spp. root rot and nematodes) and abiotic (cold) tolerance, with greater induction of dried canopy reconstitution (Guerra et al., 2014). Trees tend to be larger and vigorous, but its higher in grapefruit then in sweet orange trees, probably due to the presence or absence of viruses. 'Swingle' is a rootstock that grow well on sandy and loamy soils thanks of its moderate salinity and drought tolerance, but it's not indicate for poor and clays soils with high pH or in poorly drained areas (Wutscher, 1979; Hutchison, 1974).

Others

Some new rootstocks have been patented and released by numerous research institutes worldwide. Among these some citrandarins seem to be very interesting for their good productive behavior and tolerance to different biotic and abiotic factors.

Among the hybrids of 'mandarin Sunki' X 'Swingle trifoliate' orange, recently issued by researching community and in particular by the University of California (USA), the most promising rootstocks are:

- C22, released with the name 'Bitters', which reduces the canopy development, induces high production and tolerates tristeza and calcareous soils (Louzada et al., 2013);

- C54, released with the name 'Carpenter', which induces low vigor and high production and tolerates tristeza and calcareous soils (Siebert et al., 2010) ;

- C57, released with the name 'Furr', which induces low vigor, tolerates tristeza and calcareous soils and is more tolerant to *Phytophtora parasitica* (Louzada et al., 2013).
These selections appear promising to contribute to new citrus groves in Sicily, but their adoption as rootstocks substituting sour orange depends from their adaptability on pedoclimatic condition. There isn't, again, an 'universal' rootstock that is suitable for all conditions; in fact, research has not achieved final goals as for the genetic improvement of varieties.
In Italy in 1969, the CREA-Research Centre for Citriculture and Mediterranean Crops (Acireale) started a research program aimed at breeding citrus rootstocks using *Citrus latipes* (Swing.) as female parent and Poncirus, sour orange and Volkamerian lemon as male parents. The more interesting are the citrandarins, hybrids of *C. latipes* X *P.trifoliata* namely '**F6P12'** and '**F6P13',** respectively.
Ever since the start of citrus rootstocks experiments, all studies were aimed to use the positive properties of species botanically near to the genus *Citrus*. Based on experimental performed in greenhouse, Swingle suggested to use ***Severinia buxifolia*** (Poir) Tenore as rootstock, due to its high graft's affinity with *Citrus*. Originated from China, trees grafted on *Severinia* induced small size.

6. Agricultural techniques

The vegetative and reproductive physiology of citrus is related to a considerable number of cultural practices, depending on biotic and abiotic constraints, that develop important characters as tree development and high fruit quality yields.

Different common practices and treatments affecting flower production and commercially used to alleviate alternate bearing include pruning, girdling, defoliation, nitrogen fertilization and gibberellin application (Agustì, 2003; Guardiola et al., 1982). Interestingly, gibberellins play an inhibitory role on citrus flower bud induction and differentiation, as in many other woody trees.

Pomegranate (*Punica granatum* L.)

1. Taxonomy and origin

The pomegranate is an ancient plant consider as one of the earliest fruit species to be domesticated; the suspected progenitor of pomegranate is very similar in appearance to the domesticated form, differing mainly in the size and colour of the seeds and/or fruit (Navindra et al., 2006). The Latin name *Punica granatum* (Fig. 3.1) was given by the botanist Linnaeus and the generic name Punica refers to Pheonicia (Carthage) as a result of mistaken assumption regarding its African origin.

(adapted from Köhler, 1980)

Figure 3.1. *Punica granatum* L.

Plinio called it ‘malum punicum’ as the apple of Carthage, but during the Roman Empire it was commonly named ‘granatum’ i.e. fruit of many seeds, but many believed that the name comes from the typical colour puniceo of flower, fruit and bark. Vavilov (1951) studied the locations of the primary regions, called the ‘centers of origin of the species and variety’ for several hundreds of plants with economic importance (but excluding ornamentals and park plants), dividing on 8 center groups and establishing that pomegranate is ascribed at the fourth center that occupies the Near East, including the interior of Asia Minor, the whole of Transcaucasia, Iran, and the highlands of Turkmenistan. In fact, pomegranate plants are typical of arid and semi-arid regions due to its high adaptivity to a wide range of climates and soil conditions, becoming protagonist in the art and craft practice from the seventh century BC to the Renaissance. Pomegranate has been naturalized and domesticated since prehistoric times, starting in the Transcaucasian-Caspian region and northern Turkey (Zohary and Spiegel-Roy, 1975; Harlan, 1992). Its diffusion is estimated through colonization movements around the globe during the Roman Empire reaching the Mediterranean region, Europe, Asia and till America by Spanish sailors and Jesuit missionaries in the 1700s (Goor and Liberman, 1956; Scortichini, 1990; Barone et al., 2001; Holland et al., 2009).
Botanically, Punicaceae family contains only two species:
- *P. granatum* L., cultivated for its edible fruits,
- *P. nana* as ornamental plant (Moriguchi et al., 1987; Guarino et al., 1990).

For some authors, *Punica* genus include also *P. protopunica* Balf. f. 1882, originated and present only on the Socotra Island (Yemen), and considered as the ancestral

species of the genus (Shilikina, 1973) or an independent evolutionary path (Kosenko, 1985).

2. Economic importance and world diffusion

Current global data on production of pomegranate is unavailable in FAO statistics, because is consider as a minor fruit. However, the total worldwide production is approximated at 1,500,000 tons and Iran produces 47% of world production. In addition to Iran which has the highest area under cultivation, highest production and is the number one exporter, other countries including Turkey, Afghanistan, Pakistan, India, Armenia, Georgia, Tajikistan, Jordon, Egypt, Italy, Tunisia, Azerbaijan, Libya, Lebanon, Sudan, Myanmar, Bangladesh, Mauritania, Morocco, Cyprus, Spain, Greece, France, China, Japan, and the U.S.A. are among the countries which have areas under pomegranate cultivation. However, among these countries, India, The Central Asian Republics, Upper caucuses and Spain have the highest area under cultivation and varietals diversity (FAO, 2009). In Italy, in the last years the total area used for the cultivation of pomegranate has increased until 62 hectares and 5.131 quintals of production (Istat, 2011).

There are innumerable cultivars of pomegranate grown in the countries of origin, but the local pomegranate germplasm collections have been established in several Mediterranean countries where pomegranate is diffused. In 1934, in Turkmenistan, was established the worldwide largest pomegranate genebanks collection at the Garrygala Experimental Station for Plant Genetic Resources by Vavilov, containing over 1000 accessions of pomegranate. The collection, gathered from 27 countries on four

continents, contains plant material with economically-valuable traits and qualities that are important for breeding; these include resistance to frost and sunburn, high yield, large seeds, taste, high vitamin C content, high juice yield, thin peel, long shelf life, and resistance to pests and diseases (Turdieva, 2004). In India there are three collections containing at least 30 accessions each, and in Azerbaijan, Ukraine, Uzbekistan and Tajikistan there are a collection of 200-300 accessions. The U.S. National Clonal Germplasm Repository, in Davis, CA, hold almost 200 pomegranate accessions including many obtained from the Turkmenistan collection, distinguish several types with very soft seeds namely "seedless" (Stover and Mercure, 2007).

3. Morphological and physiological aspects

The pomegranate tree is cultivated throughout the world and is characterized for a versatile adaptivity to wide ranging climatic condition. The tree is more or less spiny and deciduous with small/narrow or oblong leaves with short stems, but in tropical and subtropical conditions it is evergreen or partially deciduous. Depending on variety, the leaves are elliptical, lanceolate or oblong, gathered in groups, opposite, without stipules, sometimes whorled, glabrous, oblong and with short petioles. The leaves colour is red in the youngest form and bright green in adulthood, while the petiole maintain its reddish colour. It may be propagated by seeds or vegetatively in the spring by hardwood cuttings, and in summer by softwood cuttings. Although the tree can survive in semiarid and arid areas without irrigation for its high drought resistance, it is very sensitive to even slight water deficit, in particular during the

sensitive phase of a plant's growth cycle as pollination and fertilization, with a consecutive reduction of the amount of fruit produced. Besides, pomegranate tree show good productivity in high salinity soils or water, and it was classified by Sánchez-Capuchino (1986) on group 4 on the resistant salinity table (Melgarejo and Salazar, 2003). Is about 38°C the optimum of temperature for fruit development, and the quality is affected by humid climate and long hot periods (Morton, 1987; Navindra et al., 2006; Sheikh, 2006).

The flowers are most commonly red to red–orange and are funnel shaped, self-pollinated or cross-pollinated by insects and are present or as single blossoms or clustered of up to five (Stover and Mercure, 2007). Botanically, the fruit is classified as a berry-like with a leathery rind (or husk) enclosing the edible portions namely 'seeds' or 'arils' that develops not from the seed-box wall but from the outer seed-coat. The fruit is globose with a diameter varying from 6.25 to 12.5 cm with a prominent distinctive feature namely 'calyx' and an hard rind. The husk is comprised of two parts: the pericarp, which provides a cuticle layer and fibrous mat and the mesocarp, which is the inner fruit wall where arils are attached. Septal membranes are the papery tissue that further compartmentalizes groups of arils. Each aril include one angular, soft or hard seed depending on sclerenchyma tissue content. The hardness and colour of rind and arils depending on variety and pedoclimatic condition (Navindra et al., 2006; Stover and Mercure, 2007).

4. Factors affecting fruit quality

4.1 Cultivar

The pomegranate is native to the subtropics and mild temperate regions of South Central Asia.
Though more than 500 cultivars of pomegranate are known around the world, today 50 cultivars of pomegranate are commonly grown because such ancient and widespread fruits often have synonymy, in which the same basic genotype is known by different names in different regions. This synonymy is related because husk and aril colour can vary markedly when grown in different regions. Some characteristics change between genotypes, and are used as 'key' to identification, consumer preference, preferred use, and potentially niche marketing (Stover and Mercure, 2007).
Evreinoff (1957) in his "Contribution à l'Etude du Granadier" reported a review that include 61 cultivars of pomegranate with greater interest in the various countries of the world, also distinguishing it in 3 groups based on the citric acid content:
- 'sugary' or 'sweet', with <0.9% of citric acid content;
-'sweet-sour', with 0.9 to 1.8% of citric acid content;
- 'acid', whit > 1.8% of citric acid content.
If the taste is a personal thing and it little can change between people, the main cultivars selected for human destiny is strictly related to the sweet flavor. For this reason, the main cultivars now released to the world are the sweet-sour 'Wonderful', 'Akko', and the sweet one 'Mollar de Elche', 'Hicanzar' and 'Bagua'.

- **Sweet cultivars**

Mollar de Elche and its selections (‘ME1’, ‘ME5’, ‘ME6’, ‘ME14’, ‘ME15’, ‘ME16’, and ‘ME17’) have Spanish origin’ where is consider the bestselling pomegranate; is very appreciated for its sweet good red fruit with soft arils. The ripen time is in October-November (Fig. 3.2)

Figure. 3.2 Mollar de Elche, Spanish pomegranate cultivar

Valenciana is an early ripening Spanish sweet variety ripen in mid-August, appreciated by consumers for the sweet taste, soft seeds and fruit present red-purple colour both in the peel than in the arils (Fig. 3.3).

Figure. 3.3. Valenciana, Spanish pomegranate cultivar

Ganesh is consider the number one pomegranate in India. It is somewhat newly developed. Fruits are large, yellowish-red and arils are sweet and soft.
Dente di Cavallo is the most important Italian cultivar, with seeds most red coloured than peel (Fig. 3.4), very appreciated for its juicy and soft tegmen.

Figure. 3.4. Dente di Cavallo pomegranate variety

Djebeli is a late ripening sweet cultivar with very large dark red fruit and with very small seed.
Primosole is a new promising accession described for the first time in 2009 by La Malfa et al., individuated in the local Sicilian germplasm, and presenting interesting properties as soft seeds, low acidity and high polyphenol and anthocyanin contents, that making this cultivar suitable for fresh and juice production and further breeding.

- **Sweet-sour cultivars**

Hicaznar is a Turkish red cultivar, considered a high producer and characterized by hard seeds.
Wonderful, with American origin, is the main commercial variety in the United States and worldwide in the last years for its attractive bright rich red fruits with sweet-tart flavor and medium soft dark red seeds (Fig. 3.5). It is a frost sensitive variety.

Figure. 3.5. Wonderful, American pomegranate cultivar

Akko is an Israeli early varieties characterize by very large (±300 g) purple red fruits, arils semi-soft seeded, dark red, sour-sweet with subtle acidic tang (Fig. 3.6).

Figure. 3.6. Akko, Israelian pomegranate cultivar

Parfianka is a variety originally imported from Dr. Gregory Levin at the Garrigala agricultural station in Turkmenistan. Parfianka is a favored selection from a collection of over 1000 pomegranate varieties and nowdays available in several nurseries. The plant is vigorous with heavy production and fruits are red and great for its soft seeds containing juice as a complex sweet-tart taste.

- **Sour cultivars**

Cagin is a cultivar originated in Malta, producing large red fruit with typically very hard and sour flavour small seed.

Patras Acide is native in Greece; the plant is extra-large and produce red fruit with very sour taste. Is consider very good for syrup.

6. Agronomic techniques

Even if pomegranate trees grow successfully in all soils, except for very calcareous or saline ones, some agronomic techniques are commonly used by farmers in order to achieve the desired configuration of the grove and of the trees and to increase the production.
Planting distances should be sufficient to ensure good lighting, allowing the fruit to fully develop their colour, and allow for the completion of other regular farming practices. Thus, greater separation between rows of trees than between trees within a row is usually adopted: 6 x 4 m, 6 x 3m, 5 x 3 m.
Irrigation is a necessary practice in pomegranate farming in arid areas where the average rainfall is not enough to

achieve a qualitatively satisfying production. Besides, the salinity of the water used for irrigation is determinant for the good fruit characteristics. Good average of total irrigation requirements for pomegranate crops are nearly 5,000 m^3 ha^{-1} (Melgarejo et al., 2010).

There are few publications about nutrient requirements and fertilization of pomegranate. Blumenfeld et al. (1998) indicate that in Israel the pomegranate is fertilized with 200-300 fertilizers units (UF) of N ha^{-1} and K_2O 200-300 UF. Besides, an excessive irrigation and nitrogen fertilization in spring can produce an imbalance favoring vegetation or flowering; excess of nitrogen, especially if is accompanied by water imbalance, may increase the cracking of the fruit before the time of maturation and it may also influence negatively on the colour development. Potassium has a favorable effect in reducing fruit cracking.

Thinning is an agronomic practice which consist in reducing fruit load at immature stage and thus allowing remaining fruits to develop to their maximum size and quality. In pomegranate, as in other fruits as peaches, apricots or loquats, this operation is performed to remove the twins, small and irregular fruits, in order to obtain fruit with size required by the market (Hueso et al., 2003; Njoroge and Reighard, 2008; Missang et al., 2011). Some pomegranate groves conduce this practice in the first week of June and should be repeated after 20-30 days (end of June and until the early of July); depending on the phenological stage of fruits at thinning, among 7-8 to 12-15 kg per tree could be removed (Melgarejo et al., 2010). After thinning, the removed fruits are left to spoil in the soil and farmers does not get any direct payback for this expensive farming practice.

The pruning is a practice used in order to increase production and the fruit quality, to favorite the production both inside and outside of the canopy, to reduce expenses of other farming practice and to facilitate their implementation (pesticides treatments, thinning and harvesting). Annual pruning should be done, and the pruning time matches the winter rest period, December-February (Melgarejo et al., 2010).

Aim of the PhD thesis

'Nutraceutical' is a term derived from 'nutrition' and 'pharmaceutics', and this term is applied to products that are isolated from herbal products, dietary supplements (nutrients), specific diets, and processed foods such as cereals, soups, and beverages that, besides for nutrition, are also used as medicine for their physiological protective benefit against chronic diseases or for supporting the functionality of the body (Kalra, 2003).

Globalization of trade, changes in consumption and food preparation, food security, fair trade, safety concerns, health trends and climate changes are considered as important factors in agribusiness and food industry. The increasing awareness of consumers on the importance of food for the nutritional and healthy properties, capable to prevent diseases, stimulates research institutes and food industries to deepen the knowledge of the overall qualities of raw materials for fresh or processed use or to design food products enriched with nutraceutical substances.

Several factors influence composition and quality of food products, and especially vegetables and fruits, in pre- and post-harvest stages, such as cultivar and rootstock, agronomical techniques and storage conditions. The possibility to enhance the synthesis of some chemical compounds, in particular flavonoids, such as phenols and anthocyanins, is an important strategy in order to obtain products with high functional activity.

The overall aim of this PhD thesis is the evaluation of agronomical and postharvest factors influencing the qualitative and nutraceutical traits of two important fruits

with a high nutraceutical potential, such as blood orange and pomegranate. In particular, these fruit were chosen for their high anthocyanin content more and more appreciated by consumers aware of its effect on human health.
For blood oranges, different aspects were evaluated, such as the influence of several rootstocks on yield precocity and fruit quality and the effect of postharvest treatments on fruit qualitative and chemical parameters.
As for pomegranate the investigation was focused on nutraceutical and physicochemical evolution observed in varieties of different provenance and on the characterization of several local Sicilian pomegranate accessions. Also a study was carried out on the gene expression analysis of anthocyanin biosynthesis during maturation stage.

EXPERIMENTAL STUDIES

Experimental study # 1

Influence of several rootstocks on yield precocity and fruit quality of two pigmented citrus cultivar

1. Introduction

In modern fruticulture the use of rootstock for most of the known species is important not only for agronomic and phytosanitary reasons but also for tree performance and fruit quality. Although the metabolic functions in a grafted plant are divided between the two plant fractions, it is well known that rootstocks greatly influence variety behaviour as it ensures provision of minerals and water for the total plant. In Citrus plants, major and minor differences have been found between species and family members; several studies have confirmed that more of horticultural characteristics are influenced by the rootstock including tree size, adaptation to certain soil conditions, photosynthesis, carbohydrate distribution, fruit yield as size, texture, internal quality and maturity harvest (Agusti et al., 2003; Castle, 1995; Davies and Albrigo, 1994 Forner-Giner et al., 2011; Machado et al., 2015; Ramin and Rezanezhad 2005; Liu et al., 2015; Martínez-Cuenca et al., 2016).

For decades, sour orange (*Citrus aurantium* L.) has been largely used in citrus industry because it was considered the most suitable rootstock in several citrus-growing areas for its good results at different pedological and environmental conditions; infact, it is well adapted to calcareous and other

soil types. Its tolerance to many fungal disease, such as *Phytophthora* spp., and to viroids such as exocortis (CEVd) and xyloporosis (CCaVd), is an important factor that brought sour orange to be widely used, especially in the Mediterranean basin. However, as a consequence of Tristeza virus (CTV) spread in many citrus areas, this rootstock cannot anymore be used in orange and mandarin orchards due to its susceptibility to this virus. Several researches in citrus-producing countries such as USA, China, Spain has been done and still are currently in order to evaluate CTV-tolerant rootstocks for the replacement/reconvertion of citrus orchards (Louzada et al. 2008; Castle, 2010; Fu et al., 2016; Legua et al., 2014).

Italy holds the 8th place in the world citrus production with about 2 million tons (FAOSTAT, 2011-2013). Half of the Italian cultivation area is concentrated in Sicily where the production of orange pigmented cultivar is relevant. 'Tarocco', 'Moro', 'Sanguinello' and 'Sanguigno' are the most important pigmented varieties and their cultivation is concentrated at the foothills of Etna volcano. Also some blond varieties are cultivated accounting for about 30 % of the whole Sicilian orange industry (Tribulato and Inglese, 2012). Among Italian blood oranges, Tarocco is the most widespread and known among consumers due to different factors, including easy peelability, good taste and higher nutraceutical properties with respect to blonde oranges, determined by the presence of anthocyanins and responsible for the attractive red brilliant colour of the pulp and of the peel (Lo Piero, 2015). In the past few years the pigmentation traits has been transferred also in other citrus fruit typologies and namely in some hybrids such as tangors, obtained through a breeding program carried out in Sicily by

CREA-ACM (Tribulato and Inglese, 2012). Sicilian typical environmental conditions, namely night/day remarkable thermal excursion, play an important role on pigment biosynthesis and accumulation on fruits of selected genotypes (Rapisarda and Giuffrida, 1994; De Pascual-Teresa and Sanchez-Ballesta, 2008; Butelli et al., 2012), thus improving nutritional value and consumer acceptance. (Reforgiato-Recupero et al., 2009; Incesu et al., 2013). Nowadays pigmented varieties represent the most valuable ones for Sicilian citrus industry and it is important for growers to find the most suitable rootstocks to be used with these selected varieties in the different pedoclimatic conditions. In this work it was evaluated the influence of several CTV resistant rootstocks on yield precocity and fruit quality of two pigmented citrus cultivar, 'Tarocco Scirè' sweet orange and of 'Mandared' tangor, respectively.

2. Materials and methods

2.1 Plant material

Two experimental fields were established in 2010 in two areas of Catania plain suited for pigmented citrus production, namely Lentini (37°17'N, 14°53'E) and Scordia (37°20'N, 14°53'E), for Tarocco Scirè and Mandared orchards, respectively. The silty-clay soil differ for pH (7.5 at Lentini and 8.5 at Scordia) and content of active lime (2% at Lentini and 3% at Scordia). The experimental design was a complete randomized block with ten replications. Tree spacing was 5 m x 3 m at Lentini and 5 m x 4 m at Scordia. The two orchards were subjected to standard cultural practices.

The cultivar evaluated were Tarocco Scirè sweet orange and Mandared triploid tangor (Nules clementine X tetraploid Tarocco orange); both these varieties are included in the list of registered citrus accessions for Italian volunteer certification program.
- Rootstocks: Troyer, Carrizo and C35 citranges, Citrumelo Swingle, Bitters (C22), Carpenter (C54), Furr (C57) (the last three are hybrids of Sunki mandarin x Swingle trifoliate orange released by the University of California Riverside in 2009), F6P12® and F6P13 (the last two are hybrids of *C. latipes* and *P. trifoliata* released by CREA-ACM in 2014) and Severinia (*Severinia buxifolia* (Poir.) Ten.).
Poncirus [*Poncirus trifoliata* (L.) Raf.] and Flying dragon (*P. trifoliata var. monstrosa*) were also evaluated in combination with Mandared, while among citranges Troyer and C35 were the only examined with Mandared.

2.2 Field and fruit quality measurements

Tree growth was monitored along 6 years; canopy volume was calculated by Turrell's formula. Yield and fruit quality were recorded since the first harvest, that started in 2013 and 2014, respectively, for Tarocco Scirè and Mandared. Number of harvested fruits, total production per plant and mean fruit weight were recorded.
Fifty fruit for each scion-rootstock combination were individually sampled and analyzed for morphological and physicochemical parameters.

2.3 Morphological and physicochemical parameters determination

Fruit height, equatorial diameter, rind thickness, weight and colour parameters were recorded on each fruit before juice extraction.

Fruit height, equatorial diameter and rind thickness (mm) were measured with an electronic digital slide gauge (Mitutoyo) with 0.01 mm accuracy; fruit weight was taken using an electronic balance (Sartorius Model BL-600) with an accuracy of 0.1 g.

Peel colour was recorded on two opposite points of the equatorial region of each fruit and juice in glass cells of 2 mm path length, using a Minolta CR-400 chroma-meter according to the international CIE L*, a*, b* values, where L* indicates lightness, a* indicates chromaticity on a green (-) to red (+) axis, and b* chromaticity on a blue (-) to yellow (+) axis. Results were expressed as citrus colour index (CCI= a*1000/L*b), widely used in the citrus industry as maturation index (DOGV, 2006).

For physicochemical and chromatographic analyses, fruits were individually squeezed with a commercial juice extractor (Kenwood Citrus Juicer JE290). Total Solid Soluble (TSS) content was determined using a digital refractometer (Atago CO., LTD, model PR-32α) and results expressed as °Brix. Titratable acidity (TA) was determined by potentiometric titration (Hach, TitraLab AT1000 Series) of the juice with 0.1 N NaOH beyond pH 8.1 according to the AOAC method (AOAC, 1995) and results were expressed as g L^{-1} of citric acid equivalent.

Vitamin C (L-ascorbic acid) was determined using an automatic titration apparatus (702 SM Titrino, Metrohm, Herisau, Switzerland) with 0.001 M I_2 and results were expressed as g L^{-1}.
For Mandared samples, total anthocyanin content (TAC) was performed spectrophotometrically by the pH differential method (Fuleki and Francis, 1968), where the absorbance was measured with a spectrophotometer (NanoDrop 2000, Thermo Scientific) at 510 and 700 nm in buffers at pH 1.0 and 4.5 and the results were expressed as mg of cyanidin-3-glucoside equivalents per liter of fresh weight, using:

$$\mathbf{A= [(A510 - A700)pH\ 1.0 - (A510 - A00)pH\ 4.5]}$$

and results expressed as mg L^{-1} of cyanidin-3-glucoside (Cy3G) concentration.
Differently, for Tarocco Scirè chemical markers were investigated for the identification of anthocyanin profile, flavanones, flavones, hydroxycinnamic acids and their derivatives.

2.4 HPLC/DAD and HPLC/ESI/MS analyses

All solvents and reagents used in this study were high purity laboratory solvents from VWR (Milan, Italy); HPLC grade water and acetonitrile were also obtained from VWR. Cyanidin 3-O- glucoside, caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid and sinapic acid, limonene and valencene were purchased from Sigma (Sigma-Aldrich., Milan, Italy), whilst neoeriocitrin, narirutin, hesperidin, didymin and vitexin were from Extrasynthese (Lyon, France).
Small portions (2mL) of the juices were put in 15 ml plastic sample tubes and 100 μL of formic acid (98%) were added.

Samples were sonicated during five minutes, then centrifuged at 4000 rpm for 15 minutes to separate the solid portion of the juices. 1 mL of the clear supernatants were transferred into 2mL HPLC amber vials and immediately analysed. Chromatographic analyses were carried out on an Ultimate3000 UHPLC focussed instrument equipped with a binary high pressure pump, a Photodiode Array detector, a Thermostatted Column Compartment and an Automated Sample Injector (Thermo Fisher Scientific, Inc., Milan, Italy). Collected data were processed through a Chromeleon Chromatography Information Management System v. 6.80. Chromatographic runs were all performed using a reverse-phase column (Gemini C18, 250 x 4.6 mm, 5 μm particle size, Phenomenex Italia s.r.l., Bologna, Italy) equipped with a guard column (Gemini C18 4 x 3.0 mm, 5 μm particle size, Phenomenex Italia s.r.l., Bologna, Italy). Polyphenols of samples were eluted with the following gradient of B (2,5 % formic acid in acetonitrile) in A (2,5 % formic acid in water): 0 min: 10 % B; 20 min: 35 % B; 25 min: 10 % B. The solvent flow rate was 1 mL min^{-1}, the temperature was kept at 25°C, and the injector volume selected was 40 μL. DAD analyses were carried out in the range between 700 and 190 nm, registering the chromatograms at 280, 330, 350 and 520 nm. Quantification was carried out at 280 nm for flavanones using calibration curves established with the corresponding analytical standards (neoeriocitrin, correlation coefficient R2 = 0.9999; narirutin, R2 = 0.9998; hesperidin, R2 = 0.9999; didymin, R2 = 0,9999). Hydroxycinnamic acids and their derivatives were quantified at 330nm using chlorogenic acid (R2 = 0.9997) as reference for cinnamoylquinic derivatives, whilst caffeic acid (R2 = 0.9998) was used for the quantification of

caffeoyl-hexose. Ferulic acid (R2 = 0.9999) was used as reference for itself and feruloyl-hexose; p-coumaric and sinapic acid were quantified using the corresponding analytical standards (R2 = 0.9999 and R2 = 0.9997, respectively). Cyanidin-3-O-glucoside (R2 = 0.9998) was used for the quantification of anthocyanins. In order to unambiguously identify the chromatographic signals and/or to confirm peak assignments, a series of HPLC/ESI/MS analyses were performed on a selected number of samples. In this case, aliquots (5 mL) of the centrifuged juices were freeze dried (Lyoquest-85, Telstar Italy, Legnano, Milan, Italy) then re-dissolved in 2 mL HPLC grade water and transferred into 2mL HPLC amber vials ready to ESI/MS analyses. ESI mass spectra were acquired by a Thermo Scientific Exactive Plu Orbitra MS (Thermo Fisher Scientific, Inc., Milan, Italy), using a heated electrospray ionization (HESI II) interface. Mass spectra were recorded operating in positive and negative ion mode in the m/z range 120-1500 at a resolving power of 25000 (full-width-at-half-maximum, at m/z 200, RFWHM), resulting in a scan rate of > 1.5 scans/sec when using automatic gain control target of 1.0 × 106 and a C-trap inject time of 250 ms. under the following conditions: capillary temperature 300 °C, nebulizer gas (nitrogen) with a flow rate of 60 arbitrary units; auxiliary gas flow rate of 10 arbitrary units; source voltage 3 kV; capillary voltage 82.5 V; tube lens voltage 85 V. The Orbitrap MS system was tuned and calibrated in positive modes, by infusion of solutions of a standard mixture of sodium dodecyl sulfate (Mr 265.17 Da), sodium taurocholate (Mr 514.42 Da) and Ultramark (Mr 1621 Da). Data acquisition and analyses were performed using the Excalibur software. All analyses were carried out in

triplicate; results are reported in milligram (mg) of compound per liter (L) of juice.

2.5 Antioxidant activity (ORAC, ABTS+ and DPPH• methods) and total polyphenols

For fruit harvested from 2015/2016, antioxidant activity (ORAC, ABTS+ and DPPH• methods) and total polyphenols (TPC) were performed on Tarocco Scirè, while ORAC and total polyphenols (TPC) for Mandared were investigated.

The ORAC assay was performed as described by Cao et al., 1993 with some modifications. The measurements were carried out on a Wallac 1420 Victor III 96 well plate reader (EG & Wallac, Turku, Finland) with a fluorescence filter (excitation 485 nm, emission 535 nm). Fluorescein (116 nM) was the target molecule for free radical attack by AAPH (153 mM) used as the peroxyl radical generator. The reaction was performed at 37 °C, pH 7.0, and Trolox (1 μM) was taken as the control standard, while phosphate buffer was used as blank. All solutions were freshly prepared prior to analysis. All samples were diluted with phosphate buffer (1:50-100, v/v) prior to analysis, and results were expressed as micromoles (μMol) of Trolox equivalents per 100 mL of juice.

For the antioxidant activity determination, a methanol extract was prepared, using 1 mL of each sample juice sample mixed with 10mL of MeOH/water (80:20, v/v)+1% HCl, and the mixture was sonicated mat 20 °C for 15 min and left for 24 h at 4 °C. Then, the extract was again sonicated for 15 min, and centrifuged at 10 000 × g for 10min. The radical scavenging activity was evaluated using

the DPPH• radical (2,2-diphenyl-1-picrylhydrazyl) method and the ABTS+ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation method. The decrease in absorbance of all samples was measured in a UV-visible spectrophotometer (Helios Gamma model, UVG 1002E; Helios, Cambridge, UK) at 515 nm and 730 nm for DPPH• and ABTS+, respectively. A calibration curve was performed with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid) (0 to 20 nmol) from Sigma (Madrid, Spain) and results were expressed as mmol of Trolox equivalent per kg of fresh weight (mmol TE kg^{-1} FW).

Total polyphenols content (TPC) was measured spectrophotometrically (ThermoSpectronic Heγios γ, England) using the Folin–Ciocalteu colourimetric method according to Singleton et al. (1999). 50 µL of each juice sample was mixed with 2.5 mL of Folin-Ciocalteu reagent (1:10 v/v), 450 µL of phosphate buffer (pH 7.8); the mixture was incubated at room temperature for 3 min and 1mL of 20% sodium carbonate was added to the mixture. The TPC was determined after 1 h of incubation at room temperature at 765 nm. Results were expressed as milligram of gallic acid equivalent per Liter of juice (mg GAE L^{-1}).

2.6 Statistical analysis

Analysis of variance (ANOVA) was carried out using STATISTICA 6.0 (Statsoft Inc.) and used to test the significance of each variable ($P \leq 0.05$). A basic descriptive statistical analysis was followed by an analysis of variance test for mean comparisons. The method used to discriminate among the means (Multiple Range Test) was Fisher's Least

Significant Difference (LSD) procedure at a 95.0% confidence level.

3. Results and discussion

3.1 Field, morphological and physicochemical measurements

The different rootstocks greatly affected several productive and vegetative parameters of the two tested varieties. The highest cumulative yields were obtained on Bitters and C35 for Tarocco Scirè, and on C35 and Furr for Mandared (Fig. 1). On the other hand Severinia and F6P13 for Tarocco Scirè and Flying dragon and Severinia for Mandared showed the lowest values of cumulative yield in the first years of production. This parameter is obviously correlated with the number of harvested fruits per plant.
Also important differences were recorded for several qualitative parameters of the fruit. As for Tarocco Scirè, higher values of fruit weight were recorded in combination with Bitters, C35 and Carrizo (Tab. 1), whereas no significant differences were recorded for Mandared (Tab. 2). Canopy volume was strongly affected by rootstock vigour showing the highest values for Furr and Carpenter, both for Tarocco Scirè and Mandared. C35 in the case of Tarocco Scirè and F6P12 in the case of Mandared also showed very high values of canopy volume. Among the less vigorous rootstocks the combinations Mandared/Flying dragon and Troyer/Tarocco Scirè exhibited the lowest values of volume. Interestingly, Bitters resulted to be less vigorous than Carpenter and Furr, as already observed by other authors (Siebert et al., 2010). As a consequence of the previous

parameters C35, along with Bitters, showed the highest yield efficiency for both Tarocco Scirè and Mandared (Tables 1 and 2).
Table 3 and Table 4 report the main physical parameters of the fruits harvested in 2014/15 and 2015/16 from Tarocco Scirè plants and in 2015/2016 from Mandared, respectively.
In the case of Tarocco Scirè the fruits of plants grafted onto Citrumelo and F6P13 showed lowest values of size in both years; as a confirm of fruit weight values referring to the whole production, the biggest fruits were recollected onto Tarocco Scirè plants grafted onto Carrizo, C35 and Bitters. *S. buxifolia* only produced in the first year of production, being all the plants grafted on this rootstock dead in the second year. Rind thickness values exhibited a great variability in the first year, likely due to the plant juvenility. In the second year rind thickness ranged from 5.0 of Citrumelo to 5.9 of C35. In 2015/16 Citrus Colour Index showed the highest values on fruits of plants grafted onto most of the Poncirus derived rootstocks (Table 3, Figure 2).
For Mandared, the highest values of fruit height were recorded with F6P13 rootstock. The values of equatorial diameter strongly varied among all the combinations ranging from 71.4 mm for Citrumelo grafted plants to 54.2 mm for C35 grafted plants. Bitters and Flying dragons determined for Mandared the highest values of fruit rind thickness while no significant differences were recorded for Citrus Colour index values (Table 4, Figure 3).
As concerning chemical parameters, both in the case of Tarocco Scirè and Mandared, several differences were recorded especially for TSS and acidity values (Tables 5 and 6). In 2015/16 the highest values of TSS:TA ratio were evidenced for Bitters, Carpenter and Carrizo on Tarocco

Scirè, and for Bitters, Furr and F6P13 on Mandared (Figure 4). Citrus juices, especially orange juice, are rich sources of ascorbic acid, which is an important antioxidant, and its content is considered as a significant indicator of orange juice quality (Arena et al., 2001). In this study, ascorbic acid content in Tarocco Scirè orange juices, did not varied significantly among the tested rootstocks whilst a slight increase of its content was noticed in fruits of the second harvest year (2015/16), being the fruits of F6P12 those with the highest content (more than 800 mg L^{-1}) (Table 5).

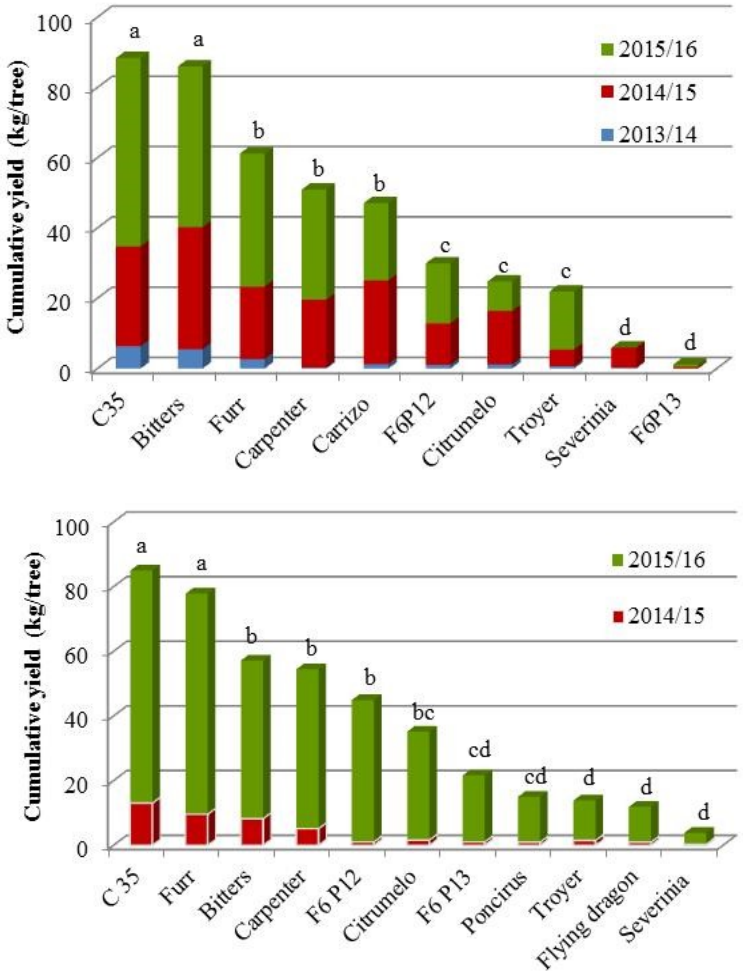


Figure 1. Cumulative production recorded on Tarocco Scirè (above) and Mandared (below) on different rootstocks.

Table 1. Vegetative and productive results of Tarocco Scirè on different rootstocks in 2015/16.

	Harvested fruits (number/tree)	Mean fruit weight (g)	Canopy volume (m^3)	Yield efficiency (kg/m^3)
Carrizo	134bcd [a]	249a	7.9bc	3.8bc
Troyer	71de	246abc	4.7d	3.5bc
C35	217a	247ab	9.4ab	5.8a
Citrumelo	41e	200e	8.5bc	1.0d
Bitters	189ab	249a	7.2c	6.4a
Carpenter	145bc	224bcd	9.5ab	3.4bc
Furr	177ab	223cde	10.1a	3.8b
F6P12	76de	240abc	7.3c	2.4c
F6P13	4cde	184de	5.1abcd	1.0bcd

[a] Values along columns with different letters are different for $P \leq 0.05$

Table 2. Vegetative and productive results of Mandared on different rootstocks in 2015/16.

	Harvested fruits (number/tree)	Mean fruit weight (g)	Canopy volume (m^3)	Yield efficiency (kg/m^3)
Troyer	81e [a]	172a	9.6de	1.2d
C35	414a	175a	9.9de	7.4a
Citrumelo	220cd	168a	11.7bcd	2.9bcd
Bitters	293abc	174a	10.8cd	4.8b
Carpenter	280bc	175a	13.5ab	3.8bc
Furr	388ab	176a	15.0a	4.7b
F6P12	259bc	179a	13.1abc	3.7bc
F6P13	128de	169a	10.1de	2.6cd
Poncirus	111de	157a	7.3d	2.4cd
Flying dragon	76e	166a	2.5f	3.8bc

[a] Values along columns with different letters are different for $P \leq 0.05$

Table 3. Physical parameters of Tarocco Scirè fruits on different rootstocks in 2014/15 and 2015/16.

	Fruit height (mm)		Equatorial diameter (mm)		Rind thickness (mm)		Citrus Colour Index	
	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
Carrizo	82.1a [a]	87.3a	79.8a	90.9a	5.4a	5.4ab	9.9abc	7.0abcd
Troyer	81.3a	82.4bc	79.5a	83.8b	5.3ab	5.7ab	9.1cde	6.7bcd
C35	79.9ab	86.1ab	76.4b	88.8ab	4.8bcd	5.9a	9.5bcd	7.5ab
Citrumelo	74.8c	79.4c	71.5d	78.4c	4.7cd	5.0b	8.4de	6.3de
Bitters	81.5a	83.1bc	78.7ab	84.2b	11.1abc	5.2ab	11.1a	7.6a
Carpenter	78.4b	89.0a	76.2bc	92.5a	4.9abcd	5.3ab	10.7ab	7.3abc
Furr	75.9c	81.4c	73.3cd	85.2b	4.5de	5.5ab	9.5bcd	7.8a
F6P12	80.5ab	82.9bc	77.8ab	85.5b	5.3ab	5.4ab	9.1cde	6.5cd
F6P13	66.8e	71.8d	62.7f	73.1d	3.0f	5.6ab	8.1e	4.2c
S.buxifolia	70.0d	-	67.3e	-	3.9e	-	5.8f	-

[a] Values along columns with different letters are different for P≤0.05

Table 4. Physical parameters of Mandared fruits on different rootstocks in 2015/16.

	Fruit height (mm)	Equatorial diameter (mm)	Rind thickness (mm)	Citrus Colour Index
Troyer	74.6b [a]	65.4bc	3.1cd	8.6a
C35	64.5d	54.2e	3.3bc	8.8a
Citrumelo	77.9b	71.4a	3.4bc	9.0a
Bitters	75.9b	62.5cd	4.0a	9.0a
Carpenter	70.2c	59.1de	2.7d	9.6a
Furr	75.8b	63.4bcd	3.4bc	9.2a
F6P12	65.4d	55.1e	3.1cd	10.5a
F6P13	81.6a	66.7abc	3.3bc	8.6a
Poncirus	76.0b	67.2abc	3.1cd	9.4a
Flying dragon	77.6b	69.2ab	3.7ab	9.1a

[a] Values along columns with different letters are different for $P \leq 0.05$

Figure 2. Tarocco Scirè fruits from plants grafted onto (from left): Bitters, C35 and Troyer citrange

Figure 3. Mandared fruits from plants grafted onto (clockwise from top left): Bitters, Carpenter, Furr and C35

Table 5. Chemical parameters of Tarocco Scirè fruits on different rootstocks in 2014/15 and 2015/16.

	TSS (°Brix)		TA (g L^{-1})		Ascorbic acid (mg L^{-1})	
	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
Carrizo	10.6abcd	10.8a [a]	1.2cde	9.1ab	495.4a	706b
Troyer	10.1bcde	10.7a	1.3bc	9.2a	484.4a	679b
C35	11.0a	9.6cd	1.2def	8.4bcd	506.8a	737b
Citrumelo	9.8cde	10.0bc	1.3bc	8.9abc	493.7a	726b
Bitters	10.9ab	10.7ab	1.2cdef	8.2cde	513.6a	745b
Carpenter	9.8cde	9.1d	1.1f	7.5e	457.9a	699b
Furr	10.6abcd	10.4ab	1.3bcd	9.1ab	461.6a	706b
F6P12	9.7e	8.9d	1.2ef	8.0de	484.7a	822a
F6P13	10.0a	10.2abc	1.4b	8.8abcd	544.4a	698b
S.buxifolia	10.1bcde	-	1.6a	-	516.2a	-

[a] Values along columns with different letters are different for P≤0.05

Table 6. Chemical parameters of Mandared fruits on different rootstocks in 2014/15 and 2015/16.

	TSS (°Brix)		TA (g L^{-1})		Ascorbic acid (mg L^{-1})	
	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
Troyer	-	11.0ab	-	13.6bc	-	652.0b
C35	14.1a [a]	12.0a	16.6a	15.5a	585.1a	678.0ab
Citrumelo	-	11.3ab	-	14.3b	-	723.1a
Bitters	13.9ab	12.0a	17.8a	12.7c	634.5a	652.0b
Carpenter	-	11.9a	-	14.5b	-	697.2a
Furr	13.4b	11.7a	16.4a	13.7bc	572.9a	634.0b
F6P12	-	11.2ab	-	14.1b	-	660.3b
F6P13	-	9.4c	-	11.1d	-	537.2c
Poncirus	-	10.8b	-	14.3b	-	657.0b
Flying dragon	-	10.6b	-	14.1b	-	646.1b

[a] Values along columns with different letters are different for $P \leq 0.05$

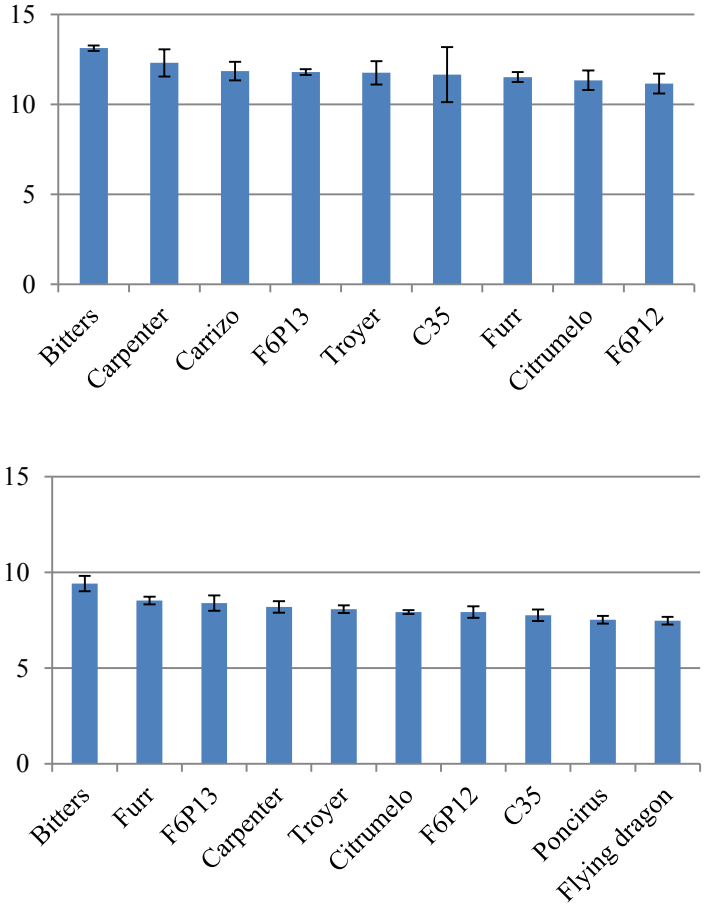


Figure 4. TSS:TA ratio recorded on fruits of Tarocco Scirè (above) and Mandared (below) grafted on different rootstocks in 2015/16.

3.2 Identification of the main chemical compounds of Tarocco Scirè orange juice

Figure 5 and Table 7 report the results of the characterization of the main chemical compound of Tarocco Scirè orange juice. This part of the work has been accomplished in order to achieve a comprehensive overview of the effects on quality of the different tested rootstocks.
A total of 23 components were tentatively identified in the juices of Tarocco Scirè object of this study. Over 23 compounds, seven belong to the subclass of anthocyanins (compounds A1-A7), three to that of flavanones (compounds F1-F3), one to that of flavones (F4), and finally 12 of them to the subclass of hydroxycinnamic acids (compounds C1-C12).
In oranges, flavanones occur mainly as glycosides, and glycosilation takes place at position 7 either by rutinose or neohesperidose. Among flavanones, hesperidin and narirutin are known as the main flavanones in orange juices, followed by didymin, neohesperidin and naringin. The most important phenolic acid in orange juice is hydroxycinnamic acid and its derivatives: ferulic, p-coumaric, sinapic, caffeic and chlorogenic acids (Rapisarda et al., 1999; Gattuso et al., 2007; Tomás-Barberán and Clifford, 2000). Table 8 reports the values of anthocyanins, flavanones and flavones and hydroxycinnamic acids measured on fruits collected on plants grafted onto observed rootstocks in 2014/15 and 2015/2016. Total anthocyanins content is greatly affected by climatic conditions; their relative values show important differences between the two years of observation, being significantly reduced in the second year, characterized by high temperatures during winter (data not shown), as also

demonstrated by the preliminary data about TAC recorded on some Mandared/rootstock combinations (Table 8). In the specific of the first year, C35, Furr and Bitters were the rootstocks that determined the presence of higher values of total anthocyanins, whilst F6P12 and Severinia were those with the lowest values. Also if the absolute values were greatly reduced, a similar pattern was observed in the second year.

As for total flavanones and flavones no important differences were recorded in the two years, being Bitters in 2014/15 and Troyer citrange in 2015/16 the rootstocks determining the highest values. These evidences about TAC and colourless flavonoids are in accordance with the findings of Crifó et al. (2011) and Lo Piero (2015) who report a whole balance of these compounds (deriving from the same pathway) and determined be several factors among which some abiotic stresses such as cold temperature play a key role.

A higher degree of variability among rootstocks was recorded for the total hydroxycinnamic acids in both years (Table 9).

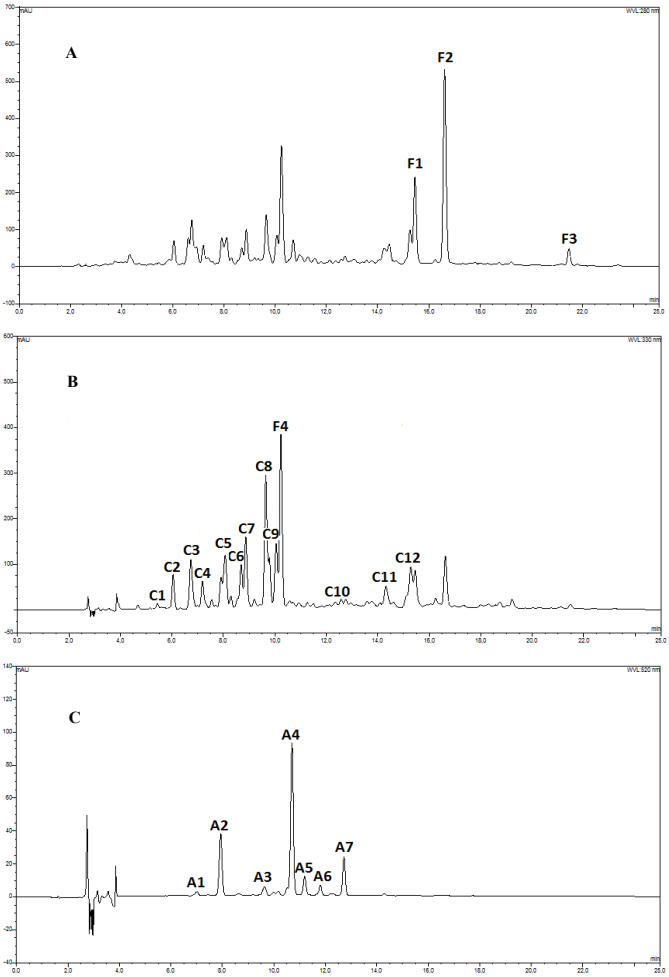


Figure 5. HPLC chromatograms, visualized at 280 (A), 330 (B) and 520 (C) nm, of Tarocco Scirè orange juice (SLT = T0). Peak letters and numbers refer to text and are listed in Table 7.

Table 7. Peak list and diagnostics for Tarocco Scirè orange juice chemical markers, as described in the text. Peak letters and numbers refer to Figure 5.

Anthocyanins - 520 nm			
	Rt, min[a]	**Compound identification**	**MW**
A1	7,09	delphinidin 3-O-glucoside	465
A2	8,17	cyanidin 3-O-glucoside[b]	449
A3	9,97	delphinidin 3-O-(6”- malonyl)glucoside	551
A4	10,90	cyanidin 3-O-(6”- malonyl)glucoside	535
A5	11,50	cyanidin 3-O-(6”- dioxalyl)glucoside	593
A6	11,80	delphinidin 3-O-glucoside derivative	465
A7	13,05	peonidin 3-O-(6”- malonyl)glucoside	549
Flavanones and flavones - 280 nm			
F1	15,29	narirutin[b]	580
F2	16,61	hesperidin[b]	610
F3	21,83	didymin[b]	594
F4	9,696	vitexin[b]	432
Hydroxycinnamic acids - 330 nm			
C1	4,48	caffeoyl-hexose	342
C2	4,97	p-coumaroylquinic acid 1[c]	338
C3	5,61	feruloyl-hexose	356
C4	5,93	p-coumaroylquinic acid 2[c]	338
C5	6,71	chlorogenic (5 caffeoylquinic) acidb + isomer	354
C6	7,23	feruloylquinic acid 1[c]	368
C7	7,46	p-coumaroylquinic acid 3[c]	338
C8	8,20	feruloylquinic acid 2[c]	368
C9	8,46	feruloylquinic acid 3[c]	368
C10	10,83	sinapic acid[b]	224
C11	12,48	p-coumaric acid[b]	164
C12	13,43	ferulic acid[b]	194

[a] as average of 3 x 10 = 30 analytical measurements;
[b] co-injection with pure analytical standards;
[c] correct isomer not determined

Table 8. Total anthocyanin content (TAC) measured on Mandared fruits on different rootstocks in 2014/15 and 2015/2016.

	TAC (mg L^{-1})	
	2014/15	2015/16
Troyer	-	3.3c
C35	15.0b [a]	5.4b
Citrumelo	-	1.1d
Bitters	16.9a	7.0a
Carpenter	-	6.9a
Furr	17.1a	2.3c
F6P12	-	1.7cd
F6P13	-	0.9d
Poncirus	-	0.8d
Flying dragon	-	1.1d

[a] Values along columns with different letters are different for P≤0.05

Table 9. Content (mg L^{-1}) of Tarocco Scirè juice anthocyanins (compounds A1-A6 in Figure 4 and Table 7), flavanones and flavones (compounds F1-F5) and hydroxycinnamic acids (compounds C1-C12) measured on fruits on different rootstocks in 2014/15 and 2015/2016.

	Total anthocyanins (A1-A6)[a] (mg L^{-1})		**Total flavanones & flavones (F1-F5) (mg L^{-1})**		**Total hydroxycinnamic acids (C1-C12) (mg L^{-1})**	
	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
Carrizo	9.4bc[b]	5.2abc	104.3de	132.4bc	112.0c	109.7de
Troyer	12.5ab	6.9a	119.7bcd	148.0a	127.1b	132.9a
C35	16.2a	2.3cd	127.5abc	129.3bc	122,4bc	112.5cde
Citrumelo	9.7bc	1.7d	101.3de	120.0c	126.3b	117.5cde
Bitters	14.8a	6.2ab	139.9a	128.5bc	121.4bc	118.8bcde
Carpenter	12.2ab	4.1abcd	136.5ab	133.3b	127.7b	122.7abc
Furr	15.1a	5.3abc	117.0cde	130.8bc	126.7b	108.8e
F6P12	6.1cd	3.7bcd	118.4bcde	136.4ab	118.9bc	122.0abcd
F6P13	12.9ab	2.7cd	106.6de	133.8b	162.5a	131.5ab
S.buxifolia	2.3d	-	100.4e	-	128.9b	-

[a] see Table 7 for legenda
[b] Values along columns with different letters are different for $P \leq 0.05$

3.3 Antioxidant activity (ORAC, ABTS+ and DPPH• methods) and total polyphenols

Different methods are commonly used for evaluating the antioxidant activity of foods, as none of them is considered fully capable for an exact determination of the total antioxidant capacity of a product. So far, different *in vitro* tests have been proposed using different classes of free radical generator or oxidant (Cao et al., 1993). Electron-transfer-based assays (ABTS and DPPH) measure the reductive capacity of an antioxidant throughout a colorimetric determination. However, ABTS takes into account both hydrophilic and lipophilic antioxidant capacity, while DPPH only considers lipophilic compounds (Kuskoski et al., 2005). The Oxygen Radical Absorbance Capacity (ORAC) assay is based on the inhibition of oxyradical-induced oxidation of 2,2'-azobis-(2-methylpropionamidine) dihydrochloride (AAPH) by substances with antioxidant properties, and it is considered by some to be a preferable method because of its biological relevance to the in vivo antioxidant efficacy (Chao et al., 2004).

In this work, total polyphenols of the juice of Tarocco Scirè were chemically determined and also three different analytical methods for determining antioxidant activity of the juice were used (Table 10).

As for total polyphenols Citrumelo and Bitters showed the highest and the lowest values (1231 and 880 mg GAE L^{-1}, respectively). The highest values of ABTS and DPPH were found in F6P13 rootstock (3.50 mmol TE kg^{-1} FW). Troyer

showed the highest ABTS values, while Bitters the lowest ones (Table 10).
ORAC assay for Tarocco Scirè did not show any statistical difference between the different rootstocks (Table 10), and range values obtained for all sample juices are in according to the recommended database for selected food of USDA (2010).
Total polyphenols and ORAC value of the juice of Mandared was also determined (Table 11). Bitters and C35 showed the highest values of TPC, whilst F6P13 exhibited the lowest values. Flying dragon evidenced the highest values of ORAC, being F6P12 the rootstock with the lowest antioxidant activity capacity.

Table 10. Antioxidant activity (ORAC, ABTS+ and DPPH• methods) and total polyphenols (TPC) measured on Tarocco Scirè fruits on different rootstocks in 2015/16.

	TPC (mg GAE L^{-1})	**ABTS** (mmol TE kg^{-1} FW)	**DPPH•**	**ORAC-value** (µmol TE 100 mL^{-1})
Carrizo	1128.33ab[a]	2.24de	2.08gh	1040.77a
Troyer	1219.24a	2.10e	4.27a	829.48a
C35	1088.18ab	2.37d	2.25f	101969a
Citrumelo	1231.36a	0.25g	1.94h	869.81a
Bitters	880.61b	2.68c	2.16fg	916.45a
Carpenter	1132.12ab	1.12f	2.59e	1030.01a
Furr	1049.54ab	3.59a	3.83b	926.45a
F6P12	990.45ab	2.20e	2.87d	1050.12a
F6P13	1191.21ab	3.48b	3.46c	762.79a

[a] Values along columns with different letters are different for P≤0.05

Table 11. Antioxidant activity (ORAC method) and total polyphenols (TPC) measured on Mandared fruits on different rootstocks in 2015/16.

	TPC (mg GAE L^{-1})	ORAC-value (µmol TE 100 mL^{-1})
Troyer	906.36ab [a]	1593.08ab
C35	960.91a	1032.61bc
Citrumelo	865.45bc	1258.02c
Bitters	979.09a	1644.34ab
Carpenter	906.36ab	1169.10bc
Furr	892.73ab	1110.87bc
F6P12	797.27cd	731.42c
F6P13	751.45d	1176.03bc
Poncirus	792.73cd	1521.88ab
Flying dragon	892.73ab	1975.10a

[a] Values along columns with different letters are different for P≤0.05

4. Conclusions

As for yield precocity, Mandared plants beared the first fruit one year later than Tarocco Scirè; this behavior is likely due the higher vigour of this hybrid that probably delayed the reachment of a balance between vegetative and reproductive growth. Almost all trees in combination with *Severinia* died in both trials probably for the high sensitivity of this rootstock to active lime levels of the soil. In this work the effect of the environment, and specifically of the low temperatures, on the juice pigmentation has been confirmed: in fact, the determination of total anthocyanin content by

HPLC analysis reveal the great difference in the two observed years, where the values were often more than doubled.

The different methods for determining antioxidant properties of the juice gave, as expected, incomparable results among them but give a first indication on the behavior of the different rootstocks. However more data seem to be necessary in order to achieve univocal interpretation on the antioxidant activity of the tested products. Also a coupling of these analytical data with evidences of in vivo tests is highly advisable.

The results herein reported indicate C35, Bitters, Carpenter and Furr as the most suitable rootstocks for pigmented oranges and hybrids in the tested conditions. These rootstocks positively affected yield precocity and enhanced fruit pulp anthocyanin content. On the other hand, some other rootstocks have to be considered as not suitable for further evaluation being their effect on qualitative fruit parameters unsatisfactory; in some cases even their survival is not possible. Soil conditions are confirmed as the most important constrains for the adoption of rootstocks alternatives to sour orange.

Experimental study # 2

Influence of postharvest treatments on qualitative and chemical parameters of Tarocco blood orange fruits to be used for fresh chilled juice

1. Introduction

Sweet oranges (*Citrus sinensis* L. Osbeck) are usually categorized into two groups according to the peel and pulp colour: blonde and pigmented (blood) oranges. Blood oranges are mainly cultivated in Sicily (Italy) where they are widely spread and play a pivotal role in local citrus industry (Barreca et al., 2016). It has been demonstrated that Sicilian typical environmental conditions (namely night/day remarkable thermal excursion) excerpt an important role on pigment biosynthesis and accumulation on fruits of selected genotypes (Rapisarda and Giuffrida, 1992; Butelli et al., 2012), thus improving nutritional value and consumer acceptance. The most important blood orange cultivars are Moro, Tarocco and Sanguinello and among these, Tarocco is appreciated for fresh consumption, especially for its easy peelability and for the low brix-acidity ratio which attenuate its sweet taste (Rapisarda and Russo, 2000). Moreover, during the last thirty years, Italian researchers have isolated a number of lines derived from old Tarocco varieties, that, on the whole, allowed widening its marketing calendar from December till May (Tribulato and La Rosa, 1994). The secondary metabolic pool of blood orange cultivars is well

known; it includes flavanone glycosides, which can be also considered as markers of the genus Citrus (Siracusa and Ruberto, 2014, and references therein), several hydroxycinnamic acids and their conjugates (Rapisarda et al., 1998; Rapisarda et al., 2009; Fallico et al., 2017), flavone glycosides (Barreca et al., 2016) and anthocyanins, to which their typical red colour is ascribable (Lee, 2002; Dugo et al., 2003; Hillebrand et al., 2004; Kelebek et al., 2008). Anthocyanin biosynthesis and accumulation mechanisms have been studied so far by several authors (Maccarrone et al., 1985, 1998; Rapisarda et al., 1994, 2001). In all the pigmented varieties the most represented anthocyanins are cyanidin 3-glucoside and cyanidin 3-(6''-malonyl) glucoside. The biosynthesis of free anthocyanins follows the flavonoid pathway and involves the expression of structural genes (responsible for enzymes directly implicated in all the metabolic reactions) and of their regulatory genes (Lo Piero, 2015, and references therein). Besides anthocyanins, other polyphenols have been investigated for their importance as quality assessment markers (Peleg et al., 1991; Rapisarda et al., 1998; Siracusa and Ruberto, 2014). In comparison to blonde cultivars, blood oranges are richer in hydroxycinnamates (Rapisarda et al., 1998; Arena et al., 2001); on the other way the presence of anthocyanins in their metabolic pool implies an higher susceptibility to chilling injury (CI), with symptoms, as peel pitting of various sizes and shapes, appearing after 2-3 weeks of storage at temperatures below 8°C (Pratella et al., 1969). Recently, cold treatment has been considered on different pigmented fruit commodities including cherry (Özkaya et al., 2015) and pomegranate (Palma et al., 2015), and blood oranges, also for flesh pigmentation enhancement

(Crifò et al., 2011). Cold treatment is also considered a reliable procedure to accomplish quarantine regulations for citrus fruit to be exported to the United States and Japan. In particular, treatment protocol T107-a (APHIS, 2006) including storage at 1.1°C, 1.67°C or 2.2°C for 14, 16 or 18 days, respectively, has been proven as effective against Mediterranean fruit-fly (*Ceratitis capitata* Wiedemann).
The renewed nutritive values of orange juice (Grosso et al., 2013; Zhuo et al., 2016) and the request of nutraceuticals by consumers are pushing towards an increase of consumption of fresh-commercial juice instead of those of other categories (from concentrate or not from concentrate). Furthermore, anthocyanins stability and nutraceutical properties are depleted by thermal processing such as pasteurization (Lo Scalzo et al., 2004, Cassano et al., 2007; Baldwin et al., 2012; Bai et al., 2013). For this reason, the extension of raw fruits shelf life could be a strategy in order to ensure the availability of fruits to be used for fresh chilled juice production during summer season.
In such a context, the aim of this work was to evaluate the effects of different postharvest storage conditions on qualitative and compositional traits of one of the latest ripening Tarocco lines, namely Tarocco "Sant'Alfio", in order to extend raw fruits availability.

2. Materials and methods

2.1 Plant material

Tarocco "Sant'Alfio" sweet orange [*Citrus sinensis* (L.) Osbeck] fruits were picked from plants grafted onto sour orange and grown in a commercial orchard located in south

east Sicily (Italy) on the mountainsides of the Etna volcano (37°17'N, 14°53'E). Fruits were harvested at commercial maturation, at the end of April, and immediately transported in laboratory.

2.2 Treatment and storage conditions

Fruits were rinsed in 3% sodium hypochlorite water solution for 10 minutes to reduce surface contamination and then dried with blotting paper. For each treatment 50 kg of fruits were used, generating three replicate samples of 15 kg of oranges, randomly packed in 3 rigid boxes, each representing one replicate.
Three different treatments were evaluated. Specifically, a first group of fruits was stored at 1±1 °C for 20 d and then at 4±1 °C and 90-95% relative humidity (RH) for 50 d (T1); a second group was stored at 4±1 °C and 90-95% relative humidity (RH) for 70 d (T2); the third group of fruits was stored at room temperature (20±1 °C) and used as control sample (CK). Three replicates of 6 healthy fruits per treatment were used, at 0, 20, 35, 48, and 70 days after harvest, for visual assessment (decay and chilling injury) and for morphological and chemical parameters determination.

2.3 Morphological and physicochemical parameters determination

Among parameters, the physicochemical determinations were recorded as reported in section 2.3 and 2.4 of "Experimental study #1".

Furthermore, the fruit firmness was tested using a texture analyser (TA.XT2 texture analyzer, Godalming, UK) equipped with a flat compression plate; fruit resistance to a compression of 10 mm was expressed in Newton (N).

2.4 HPLC/DAD and HPLC/ESI/MS analyses

Anthocyanin profile, flavanones, hydroxycinnamic acids and their derivatives were recorded as reported in section 2.4 of "Experimental study #1".

2.5 GC/MS analyses

3 mL of each samples was conditioned for 10 min at 40 °C in a sealed vial in a thermostatic bath. A Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) fiber (Sigma-Aldrich, Milan, Italy) was inserted into the vial and exposed for 2 cm to the vial head space for 30 min. The volatile compounds were desorbed by inserting the fibre into the gas chromatograph injection port for 10 min at 250 °C.
Gas chromatographic (GC) analyses were run on a Hewlett-Packard gas chromatograph mod. 5890, equipped with a flame ionization detector (FID). GC-FID analyses were carried out with the following analytical conditions: Zebron ZB-5 capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness); helium as carrier gas; injection in splitless mode; injector and detector temperatures 250 and 280 °C, respectively. The oven temperature was programmed as follow: 40 °C for 12 min, from 40 to 180 °C at 3 °C/min, after 2 min from 180 to 200 °C at 5 °C/min and the end temperature maintained for 3 min. Gas chromatography-mass spectrometry (GC-MS) was carried out on the same

gas chromatograph connected to a Hewlett-Packard mass spectrometer model 5971A, ionization voltage 70 eV, electron multiplier 1700 V, ion source temperature 180 °C, mass spectra data were acquired in the scan mode in m/z range 40-400. Gas chromatographic conditions were the same as above.
The identification of components was based on their GC retention index (relative to C9-C22 n-alkanes on the ZB-5 column), computer matching of spectral MS data with those from Wiley 275 library, the comparison of the fragmentation patterns with those reported in the literature (Adams, 2007) and, whenever possible, co-injections with authentic samples.

2.6 Statistical analysis

The statistical analysis was carried out as reported in section 2.6 of "Experimental study #1".

3. Results and discussion

3.1 Effects of treatments on decay, morphological and physicochemical parameters during shelf life test

As expected, the main effect of cold treatment imposed to Tarocco "Sant'Alfio" sweet orange was observed on decay. In fact, at the end of storage period fruit decay percentage was less than 12% for both T1 and T2 whereas the percentage of fruits affected by fungal spoilage diseases (blue and green molds by *Penicillium italicum* Wehmer and *P. digitatum* Sacc.) reached 43.3 % in the control (**Table 1**).

Table 1. Decay percentage after 70 days, Citrus Colour Index, fruit diameter and height of Tarocco Sant'Alfio oranges at 0 and 70 days after harvest and storage. Fruits were stored at 1±1°C for 20 days and 4±1°C for 50 days (T1), at 4±1 °C for 70 days (T2) and at 20±1 °C for 70 days (control, CK).

	Decay (%)	Citrus Colour Index		Fruit diameter (mm)		Fruit height (mm)	
	70 d	**0 d**	**70 d**	**0 d**	**70 d**	**0 d**	**70 d**
CK	43.3±7.3[a]	5.3a[b]	7.2a	86.5a	78.0b	83.7a	73.7b
T1	11.3±6.1	5.4a	5.1b	83.2a	83.1a	80.5a	79.5a
T2	10.0±1.4	5.2a	5.4b	84.9a	84.0a	85.6a	81.8a

[a] Standard deviation (n=3)
[b] Values along columns with different letters are different for P≤0.05

Weight losses of fruits subjected to both cold storage conditions T1 and T2 were significantly lower than in fruits stored at 20 °C. This trend, markedly evident from day 20, continued until the end of the storage period when the weight of fruits held continually at 20 °C declined to 82.7 of the initial, while losses in cold stored fruits in both treatments never exceeded 5 % (Fig. 1).

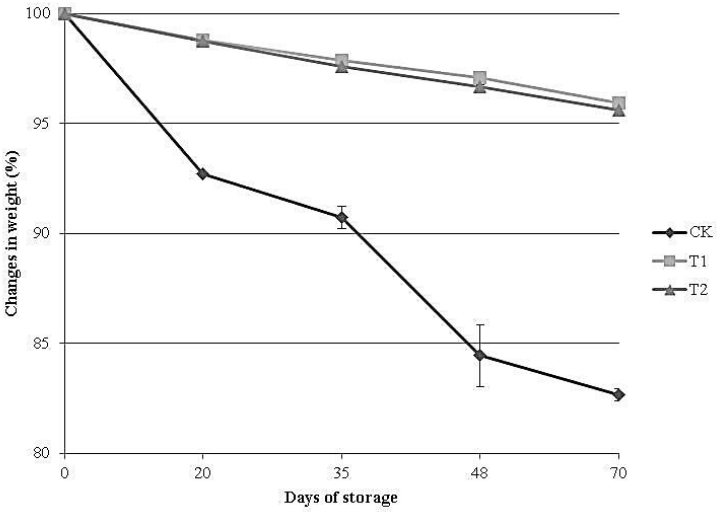


Figure 1. Weight changes (%) of Tarocco Sant'Alfio blood oranges stored at 1±1 °C for 20 days and 4±1 °C for 50 days (T1), at 4±1 °C for 70 days (T2) or at 20±1 °C for 70 days (control, CK). Vertical bars represent the standard deviation (n=6).

Chilling injury symptoms, gradually developed on the peel, did not affect fruit internal quality. Cold storage regimes were very effective in preserving fruit firmness. In fact, at the end of the storage period, control fruits exhibited the lowest firmness values (26.7 N) as compared to the values registered for T1 and T2 fruits, 46.6 and 45.2 N, respectively (Fig. 2).

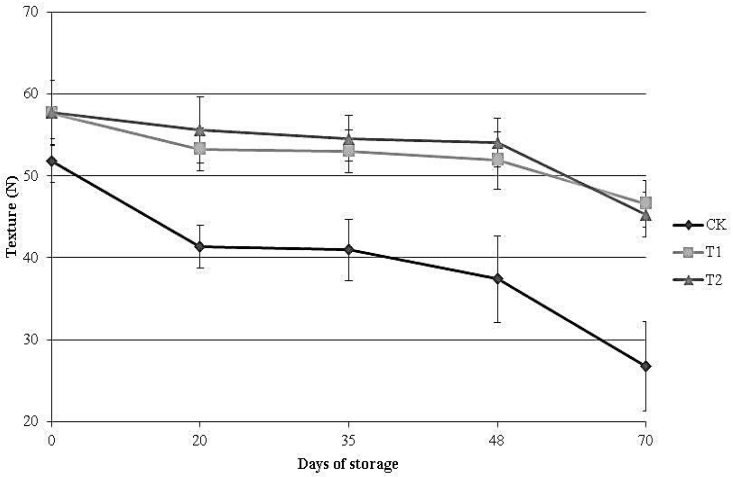


Figure 2. Fruit texture (N) evolution of Tarocco Sant'Alfio blood oranges during 70 days of storage. Fruits were stored at 1±1°C for 20 days and 4±1°C for 50 days (T1), at 4±1 °C (T2) or at 20±1 °C (control, CK). Vertical bars represent the standard deviation (n=6).

Juice red colour is one of the most important parameters for fruit quality of blood oranges and recently Lo Piero et al. (2015) reported as cold treatments can increase juice anthocyanin content.

In our conditions, at the end of storage period, control fruits exhibited a significant higher value of CCI (Tab. 1) than harvest time for the concomitant decrease of L* and increase of a* values, whereas no relevant changes occurred in cold treated fruits. Concerning the juice chemical parameters, TSS content appears to be mostly related to the weight loss, being higher on control fruits where it reached 12.5 °Brix after 70 days; at the same date, TSS values in T1

and T2 were 11.6 and 11.5 °Brix, respectively. A similar behavior was observed for vitamin C, for which weight losses determined its slight increment. Titratable acidity did not show significant differences among treatments (Tab. 2).

Table 2. Total soluble solids (TSS), titratable acidity (TA), and vitamin C content of Tarocco Sant'Alfio juice at 0 and 70 days after harvest and storage. Fruits were stored at 1±1°C for 20 days and 4±1°C for 50 days (T1), at 4±1 °C for 70 days (T2) and at 20±1 °C for 70 days (control, CK).

	TSS (°Brix)		TA (g L^{-1})		Vitamin C (g L^{-1})	
	0 d	**70 d**	**0 d**	**70 d**	**0 d**	**70 d**
CK	11.1a[a]	12.5a	10.1a	8.9a	671.9a	856.2a
T1	11.0a	11.6b	10.0a	8.5b	668.8a	745.2b
T2	10.9a	11.5b	10.7a	8.0b	651.3a	748.8b

[a] Values along columns with different letters are different for P≤0.05

3.2 Identification of the chemical markers in Tarocco orange juice

A total of 23 components were tentatively identified in the juices of Tarocco "Sant'Alfio" object of this study (Fig. 3; Tab. 3); these compounds have been used herein as chemical markers to evaluate differences and similarities all throughout the analytical batch. Over 23 compounds, six belong to the subclass of anthocyanins (compounds A1-A6), four to that of flavanones (compounds F1-F4), one to that of flavones (F5), and finally 12 of them to the subclass of hydroxycinnamic acids (compounds C1-C12).

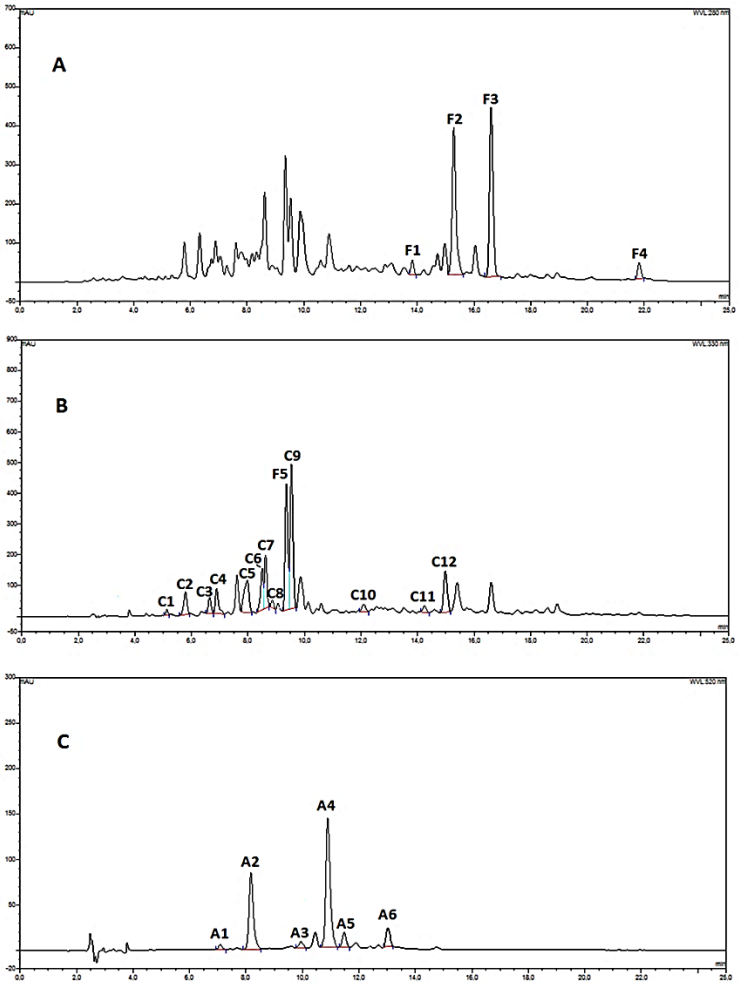


Figure 3. HPLC chromatograms, visualized at 280 (A), 330 (B) and 520 (C) nm, of Tarocco Sant'Alfio blood orange juice (SLT = T0). Peak letters and numbers refer to text and are listed in Table 3.

	Rt, min[a]	Compound identification	λmax, nm[b]	MW	ESI+/ESI- data, m/zc
Anthocyanins					
A1	7,09	delphinidin 3-O-glucoside	524, 320sh, 278	465	465 (M)+, 303*
A2	8,17	cyanidin 3-O-glucoside[d]	515, 278	449	449 (M)+, 287*
A3	9,97	delphinidin 3-O-(6”- malonyl)glucoside	520, 328sh, 284	551	551 (M)+, 465*
A4	10,9	cyanidin 3-O-(6”- malonyl)glucoside	517, 330sh, 279	535	535 (M)+, 449*, 287
A5	11,5	cyanidin 3-O-(6”- dioxalyl)glucoside	517, 278	593	593 (M)+, 449*, 287
A6	13,05	peonidin 3-O-(6”- malonyl)glucoside	518, 330sh, 278	549	549 (M)+, 463*, 301
Flavanones and flavones					
F1	14,2	neoeriocitrin[d]	328, 284	596	595 (M-H)-
F2	15,28	narirutin[d]	329, 283	580	579 (M-H)-,433*,271
F3	16,61	hesperidin[d]	326, 284	610	609 (M-H)-, 463*, 301
F4	21,82	didymin[d]	328, 283	594	593 (M-H)-
F5	9,69	vitexin[d]	339, 270	432	431 (M-H)-*, 311

[a] as average of 3 x 5 x 3 = 45 analytical measurements;
[b] from HPLC;
[c] main peaks marked with an asterisk;

Hydroxycinnamic acids					
	Rt, min[a]	**Compound identification**	**λmax, nm[b]**	**MW**	**ESI+/ESI- data, m/zc**
C1	5,32	caffeoyl-hexose	328, 330sh	342	341 (M-H)-*,179
C2	5,99	p-coumaroylquinic acid 1[e]	312	338	337 (M-H)-*,191
C3	6,89	feruloyl-hexose	326, 300sh	356	355 (M-H)-*, 193
C4	7,15	p-coumaroylquinic acid 2 [e]	313	338	337 (M-H)-,191*
C5	8,17	chlorogenic (5 caffeoylquinic) acid[d] + isomer	325, 298sh	354	353 (M-H)-*,191
C6	8,81	feruloylquinic acid 1[e]	323, 300sh	368	367 (M-H)-,191*
C7	8,92	p-coumaroylquinic acid 3[e]	313	338	337 (M-H)-,191*
C8	9,17	feruloylquinic acid 2[e]	322, 300sh	368	367 (M-H)-*,191
C9	9,85	feruloylquinic acid 3[e]	324, 300sh	368	367 (M-H)-,191*
C10	12,46	sinapic acid [d]	324	224	223 (M-H)-
C11	14,58	p-coumaric acid [d]	310	164	163 (M-H)-
C12	15,31	ferulic acid [d]	323, 295sh	194	193 (M-H)-

[d] co-injection with pure analytical standards;
[e] correct isomer not determined.

Table 3. Peak list and diagnostics for Tarocco Sant'Alfio orange juice chemical markers, as described in the text. Peak letters and numbers refer to Figure 3.

The presence of anthocyanins (mainly cyanidin derivatives) in blood oranges is broadly reported in literature, as already stated in the introduction section; same for flavanones narirutin, hesperidin (as main compound) and dydimin (Barreca et al., 2016; Rapisarda et al., 2009). We also found neoeriocitrin (compound F1), even in small amounts, whose identity has been confirmed by its spectral data and co-injection with the corresponding standard. Barreca et al. (2016) reported a series of flavones in blood orange, we found vitexin (apigenin 8-C-glucoside, compound F5) as the sole flavone present in detectable amounts in our matrices; this assignment was corroborated by spectral data and co-injection with the pure commercial compound. As regarding hydroxycinnamic acids, it is known citrus fruits, including blood oranges, contain these molecules in their free and conjugated form (Peleg et al., 1991; Fallico et al., 1996; Rapisarda et al., 1998). Nevertheless, the majority of authors prefer to report the content of the four main hydroxycinnamic acids (caffeic, ferulic, p-coumaric and sinapic) in their free form after performing a mild hydrolytic procedure. Tounsi et al. (2010) reported the presence of chlorogenic (5-caffeoylquinic) acid in blood orange juices in considerable amounts. No data are currently available on free and conjugated hydroxycinnamic acid profile in blood orange juices. Mass spectrometric data were particularly helpful in the tentative identification of peaks C1-C12, all showing similar or even nearly identical UV-Vis spectra, typical of that of the subclass of hydroxycinnamic acids (Tab. 3). Extraction of ion at m/z = 191 (quinate ion) from the TIC (total ion current) chromatograms, diagnostic for hydroxycinnamoylquinic acids, allow to locate all peaks belonging to this particular subclass; analysis of the

corresponding mass spectra gave us the possibility to tentatively identify three p-coumaroylquinic acids (C2, C4, C7, with a pseudomolecular ion at m/z = 337) and three feruloylquinic acids (C6, C8, C9, with a pseudomolecular ion at m/z = 367). We have also identified peak named C5 as chlorogenic acid co-eluting with an isomer (pseudomolecular ion at m/z = 353). Peaks C1 and C3 (pseudomolecular ions at m/z = 341 and m/z = 355, respectively) showed in their mass spectra the diagnostic ions at m/z = 179 (C1) and m/z = 193 (C3), corresponding to the loss of an hexose; they have therefore been tentatively identified as the hexose-conjugated forms of caffeic and ferulic acid (pseudomolecular ions and fragments have been assigned according to Clifford et al., 2006, 2007).

3.3 Effects of treatments on chemical markers during shelf life test

The chemical markers identified (see previous paragraph) were gathered according to their corresponding polyphenol subclass (anthocyanins, flavanones and flavones, hydroxycinnamic acids) and monitored all throughout the analytical batch in search for differences based on the treatment applied during the shelf life test. As shown in Fig. 4 (for polyphenol content for individual phenolic subclasses, see Supplementary Table 4), juice anthocyanin content underwent a dramatic change from harvest (T0) to the end of shelf life period (T70) only for cold treatment T2, as the value raised up from 7.11 mg L^{-1} to 54.44 mg L^{-1}. At the same date (70 days after harvest), juice anthocyanin content was 11.83 mg L^{-1} in CK and 11.12 mg L^{-1} in T1. This is likely due to the physiological response of the fruit to the

cold storage conditions imposed, as already reported in literature (Crifò et al., 2011). Interestingly juice anthocyanins from fruits subjected to the colder temperature (1 °C) for 20 days (T1), did not exhibit any increment at the end of storage period, suggesting a possible inhibition of anthocyanin biosynthesis and accumulation at very low temperatures. Actually, the adoption of temperature regimes below 4 °C was considered in this study with the aim of increasing anthocyanin content in fruits to be used for juice production, being on the other hand well known the negative effect of such a low storage temperature on peel fruit (Lado et al., 2014).

As regarding the content of the other two subclasses considered, that is, flavanones and flavones and hydroxycinnamic acids, no univocal trend was observed (Fig. 4). In fact, these metabolites showed ups and downs throughout the whole storage period for all the treatments applied; in our opinion this is due to the balance of two antithetic processes occurring in the fruit: the degradation process on one side and the physiological response to stress on the other, which usually activates the biosynthesis of defense molecules (Siracusa and Ruberto, 2014). This is particularly true for cold treatments T1 and T2 for which the same weight loss was registered (see paragraph 3.1). The irrelevance of degradation processes for hydroxycinnamic acids (Fig. 4) is confirmed by the absence of off-flavour products in the volatiles (see next paragraph). Taken as a whole, these data suggested that the response of the fruits to cold (within certain limits) is mainly charged to anthocyanins rather than to the other phenolic subclasses considered.

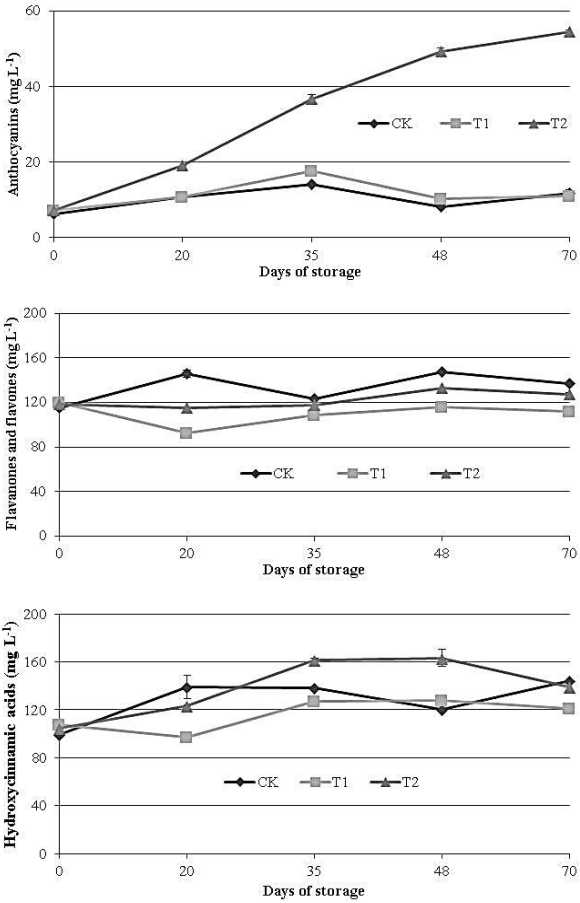


Figure 4. Content (mg L^{-1}) of Tarocco Sant'Alfio juice anthocyanins (compounds A1-A6 in Figure 3 and Table 3), flavanones and flavones (compounds F1-F5) and hydroxycinnamic acids (compounds C1-C12) during 70 days of storage at 1±1 °C for 20 days and 4±1 °C for 50 days (T1), at 4±1 °C for 70 days (T2) or at 20±1 °C for 70

days (control, CK). Vertical bars represent the standard deviation (n=3).

3.4 Aroma evaluation during shelf life test

At T0 fruits of the three treatments showed a similar volatiles profile dominated by the high amount of limonene (80-98%) followed by valencene (1.3-4.4%). All other compounds were below 1% with the unique exception of mentha-1,4,8-triene present only in CK with a percentage of 1.9%. With this starting situation our attention was focused on the potential decrease of these two main compounds and the contemporary appearance and increase of hydroxycinnamic acids degrading products such as *p*-vinylguaiacol and *p*-vinylphenol responsible for juices organoleptic decay (Fallico et al., 1996). At the end of storage period (70 days from harvest) no evident changes in volatile profiles of all samples were observed (data not shown). Despite a substantial unchanged relative percentage of limonene and valencene, only a slight increase for minor compounds such as β-myrcene and neryl acetone up to 1.7 and 2.3%, respectively, was observed. No traces of vinylphenols were recorded, in accordance with HPLC data showing a good stability of cinnamic acids for all samples during the full observation period.

4. Conclusions

Cold regimes represent the most common postharvest technology to effectively prolong fruit life and reduce decay development. For sweet oranges cold treatment are also compulsory for accomplishing importing rules in certain

countries (i.e. for Mediterranean fruit fly). In the case of Tarocco blood oranges, mostly devoted to fresh consumption, cold treatments are also useful for pigmentation enhancement, as anthocyanins biosynthesis is known to be activated as a response to thermal stress.

In this work two different cold storage protocols were tested on a very late Tarocco line in order to assess the feasibility to prolong the market window until the early summer for its use in fresh juice processing.

The tested cold storage protocols reduced fruit decay incidence to reasonable values (less than 12%), limited weight loss, did not hamper internal fruit quality, and, in the case of T2, induced a relevant increase in total anthocyanin content. In the case of T1 the storage temperature of 1 °C imposed during the first 20 days of storage was confirmed as negative for the damages determined on fruit peel (data not shown) and was also ineffective in determining an enhancement of anthocyanin content.

No relevant changes in the volatile profiles were observed for all three cold storage conditions.

On the whole, our results suggest that in the case of products expected to have high healthy properties determined from the high content of antioxidant compounds (such as blood oranges chilled juice), cold treatments of raw fruit may represent a useful strategy to guarantee the availability of fresh-high-quality juice, far from the harvest season. However the temperature regimes to be applied must take into account the inhibitive effect of extremely low temperatures, even as elicitor treatment, at least on the genotype considered in this study. In such a picture, the set-up of cultivar-specific protocols and their tuning would be advisable for blood orange industry and for the possibility to

further prolong the presence of such a high valuable product on the market, at least for fresh-chilled juice production.

Experimental study # 3

Nutraceutical and physicochemical characteristics of pomegranate fruits (*Punica granatum* L.) in two Mediterranean areas and their evolution during maturation stage.

1. Introduction

Pomegranate (*Punica granatum* L.) is an appreciated ancient species, deeply embedded in many human cultures since its organs, mainly fruits, are appreciated and also used for their medical properties (Seeram et al., 2006).
Pomegranate and its derived products are more and more used for their antioxidant activity and health-promoting effects for reducing the risk of cancer, of cardiovascular disease, diabetes, Alzheimer's, infant brain ischemia, male infertility, obesity, arthritis and colitis (Bhandari, 2012; Jurenka, 2008; Kasimsetty et al., 2010; Lansky and Newman, 2007; Miguel et al., 2010).
Apart from the juice, extracts of all parts of the fruit appear to have therapeutic properties due to the presence of ellagic acid, ellagitannins including punicalagins, punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonols and flavones (Kasimsetty et al., 2010).
The pomegranate germplasm is very vast, due to the presence of a huge number of local varieties and cultivars in each growing country; generally speaking pomegranate varieties are classified according to the taste of the juice (sweet, semi-sour or sour), to the colour of the skin, and to

the seeds hardness (Hasnaoui et al., 2011). Several studies have confirmed that cultivar, pedoclimatic condition, growing region and maturity status affect the organoleptic, nutritional and functional quality related to the accumulation of some chemicals. Among the others, colour, flavor, aroma, firmness and appearance are the most important factors affecting consumer preference, even if the quick increase in demand of this product depends on nutritional and functional components, including sugars, lipids, proteins, organic acids, minerals, phenols, carotenoids and vitamins content (Gil et al., 1995 a,b; Hernández et al., 2012; Kulkarni and Aradhya, 2005; Serrano, 2012). The attractive reddish colour of arils is associated with its antioxidant activity due to the incidence of anthocyanins, a water-soluble polyphenolic pigment very sensitive to environmental condition; infact, it was demonstrated that high temperature reduce anthocyanin accumulation in peel and arils for the inhibition of mRNA transcription of anthocyanin biosynthesis genes (Shwartz et al., 2009; Borochov-Neori et al., 2009, 2011).

On the whole, little information is available on the evolution of the main nutritional and functional elements, including minerals, along maturation process; some studies have observed that pomegranate fruit is a good source of minerals and content variation of these compounds could originate from cultivar, soil and pedoclimatic conditions. Generally, potassium is the major element present in the whole fruit; during fruit development there is an increases in accumulation of macronutrients as potassium, sodium and calcium in arils and juice with decreasing of magnesium, sodium, calcium and micronutrients (Al-Maiman and

Ahmad, 2002; Fawole and Opara, 2013 a,b; Mirdehghan et al., 2007).

2. Morphological and physicochemical analysis

Fruits of Mollar de Elche (MOL), Valenciana (VAL) and Wonderful (WON), well known pomegranate varieties, were obtained from plants located at the experimental field stations of Miguel Hernández University in the province of Alicante, Spain (02°03'50''E, 38°03'50'' N) and of University of Catania in the province of Catania, Italy (15°03'16'' E, 37°24'37'' N). The three considered cultivar were additionally coded with "IT" and "ES" according to the place of cultivation, Italy or Spain, respectively. The plants of the three cultivars were included into pilot plantations and were subjected to standard cultural practices. For each plantation, the main meteorological parameters were recorded.

Morpho-pomological measurements and chemical analyses were carried out on samples of 10 mature fruits per genotype selected at random throughout the external and internal canopy in the four cardinal directions. Three harvest times in 2015 (21 September, 6 and 21 October) were considered in order to evaluate, for each accession, the maturation pattern. Fruits were carefully cut in half and arils extracted by hand, and juice was obtained using a commercial juice extractors. The juice was used to determine the principal chemicals Chemical composition and the antioxidant activity were determined on the fresh squeezed juice. The moisture (M) percentage of arils was determined after being dried in a hot air oven at 60 °C until reaching a constant weight; three repetitions per variety

were carried out. Then, the dried arils were milled and used for minerals composition.

2.1 Fruit weight, size and colour measurements

The peel colour fruits was instrumentally evaluated using a Minolta C-300 Chroma Meter (Minolta Corp., Osaka, Japan) coupled to a Minolta DP-301 data processor. This colourimeter uses an illuminant D65 and a 10° observer as references. Colour was assessed according to the Commission Internationale de l'Éclairage (CIE) and expressed as L*, a*, b*.
Fruit weight (FW) (g) was determined using an electronic balance (Sartorius model BL-600, Madrid, Spain) with an accuracy of 0.1 g; equatorial diameter (D1) (mm), fruit length without calyx (L1) (mm) were measured with an electronic digital caliper (model CD-15 DC; Mitutoyo (UK) Ltd, Telford, UK) with 0.01 mm accuracy.

2.2 Analysis of organic acids and sugars

Individual organic acids and sugar profile were determined according to Legua et al., 2012. Twenty milliliters of pomegranate juice were centrifuged at 10,000 g for 20 min (Sigma 3-18K, Osterode and Harz, Germany) and the supernatant was filtered through a cellulose nitrate membrane filter (0.45 m pore size). Then, samples were injected (10 μL) into a Hewlett-Packard HPLC series 1100 (Wilmington DE, USA) with an autosampler and an UV detector coupled with a refractive index detector (HP 1100, G1362A). The elution system consisted of 0.1% phosphoric acid with a flow rate of 0.5 mL min^{-1}. Organic acids were

isolated using a Supelco column [Supelcogel TM C-610H column (30 cm×7.8 mm), i.d., Supelco, Bellefonte, PA, USA] and Supelguard C610H column (5 cm×4.6 mm, Supelco, Inc.). The absorbance was measured at 210 nm using a diode-array detector (DAD), and results were expressed as g 100 mL^{-1}. These same HPLC conditions (elution buffer, flow rate and column) were used for the analysis of sugars. The detection was conducted using a refractive index detector (RID). Standard curves of pure organic acids (oxalic, citric, tartaric, malic, quinic, shikimic, and fumaric acids) and sugars (glucose, fructose and sucrose) were used for quantification. Sugar and organic acid standards were obtained from Sigma (Poole, Dorset, UK).

Total Soluble Solids (TSS), titratable acidity (TA) and total anthocyanin content (TAC) were determined according to the methods reported in section 2.3 of "Experimental study #1".

2.2 Antioxidant activity (ABTS+, DPPH• and FRAP methods) and total polyphenols

Methods (Benzie and Strain, 1996; Re et al., 1999; Singleton et al., 1999) used for the antioxidant activity determination were used with some modification in the reaction time as reported in section 2.5 of "Experimental study #1.". Additionally, the ferric reducing antioxidant power (FRAP) was also employed. Briefly, 10 µL of the supernatant were mixed with 990 µL of FRAP solutions and placed under dark conditions for 10 min, and the decrease in absorbance of all samples was measured in a UV-visible spectrophotometer (Helios Gamma model, UVG 1002E;

Helios, Cambridge, UK) at 515 nm for DPPH•, at 734 nm for ABTS+ and 593 nm for FRAP. Results were expressed in mmol TE kg^{-1} of fresh weight.

2.3 Mineral analysis

Approximately 1 g of milled dried arils of each sample were added with 5 mL of concentrated HNO_3, 65% (w/v), and digested for 3 h a temperature below 130 °C, in a multi-place digestion block, Selecta Block Digest 20 (Selecta, Barcelona, Spain). Samples were left to cool down to room temperature, transferred to volumetric flask and dilutions 1:10 and 1:50 were prepared using ultrapure deionised water, 18 MΩ (Milli-Q® system; Millipore Corporation, Madrid, Spain). Determination of macro-nutrients (Ca, Mg, and K) and micro-nutrients (Cu, Fe, Mn, and Zn) in previously mineralized samples was performed using a Unicam Solaar 969 atomic absorption-emission spectrometer (Unicam Ltd, Cambridge, UK). All minerals were analysed using atomic absorption except K, which was measured using atomic emission. In each analytical batch, at least one reagent blank and one spike were included to assess precision and accuracy for chemical analysis. Calibration curves were used for the quantification of minerals and showed good linearity (R2 ≥0.999). Analyses were run in triplicate.

2.4 Statistical analysis

The statistical analysis was carried out as reported in section 2.6 of "Experimental study #1".

3. Results and discussion

3.1 Morphological and physicochemical analyses

Fruit weight and size of the fruits of different accessions cultivated either in Italy and in Spain are reported in Table 1. In the considered interval a relevant increase of fruit size was evidenced for all the tested varieties independently from the cultivation area. The values showed as Wonderful was the cultivar with bigger fruits independently from its provenance.

As well known peel colour is considered an important quality attribute that influences the consumers' choices and preferences. Colour parameters displayed statistically significant differences among samples during the three harvest times (Table 2). On the basis of harvest times, for all samples a decrease of L* (lightness), b* (yellowness) and hue angle and a slight increase or a* (redness) was observed. Interestingly, Valenciana was the first variety showing a pronounced red coloration in September, while the other two varieties reached the similar a* values only at the third harvest in late October, with the exception of the cultivar Wonderful cultivated in Spain. A general increase of the green-red coordinate a* until the end of October was recordered for all Italian varieties, probably due to the latest increase on biosynthesis and accumulation of anthocyanin pigments related to the ripening time. Regarding chrome (C*), which represents the colour intensity and used to determine the quantitative attribute of colourfulness, no significant differences among samples during the three harvest times were observed.

As concerning the chemical values of the juice (Table 3), Valenciana confirmed to be the first variety to reach satisfactory values of maturation indexes. The sweet-sour variety Wonderful (appreciated for the deepness and uniformity of red colour of its fruit), at the first harvest exhibited similar TSS content to that of Valenciana, suggesting that its sweet-tart flavor is likely due to the higher levels of TA (13.2 and 15.7 g L^{-1} for the Italian and Spanish samples, respectively) as compared to all the other tested varieties (always below 3.0 g L^{-1}).
The moisture (M) percentage did not show any difference between the different cultivars during the different harvest times (data not shown).

3.2 Individual organic acids and sugar

The composition and concentration of organic acids are important factors to determine consumer perceptions of both sweetness and sourness in pomegranate fruit cultivars (Holland et al., 2009). The citric acid is the major acid accounting for titratable acidity in pomegranate fruits and its amount decrease with advancing of maturity stages (Melgarejo et al., 2000; Shwartz et al., 2009).
The results of our analyses revealed several differences of organic acids content between tested cultivars during the harvest times; malic, quinic and citric were the main organic acids detected (Table 5), and trace of phytic acid were found (data not shown).
As for the sugars detected by HPLC, fructose and glucose were the most abundant in pomegranate juices, being the first almost double than the second one (Table 5). These results confirm the predominance of fructose and glucose as

the main pomegranate sugars, in agreement with previous works (Al-Maiman and Ahmad, 2002; Fawole and Opara, 2013a; Hasnaoui et al., 2011; Legua et al., 2012-2016; Melgarejo et al., 2000; Mena et al., 2011; Shwartz et al., 2009; Tezcan et al., 2009). Also different authors report that divergences noticed on sugar contents of pomegranate cultivars might be due to different agro-climatic conditions and genotype (Hasnaoui et al., 2011; Melgarejo-Sánchez et al., 2015; Mphahlele et al., 2014).

3.3 Antioxidant activity (ABTS+, DPPH• and FRAP methods) and total polyphenols

The different methodologies adopted were not consistent in the data interpretation. Factors such as considered genotype and different maturation period of each cultivar might account for the divergence observed. On the whole an increase of the antioxidant activity was recorded in all cultivars tested during the three harvest periods (Table 4).
The analysis of phenols by means of the Folin-Ciocalteu assay provides valuable information for evidencing varieties with a higher antioxidant potential. In fruits, phenols are associated with colour, sensory characteristics (flavor, astringency and hardness), nutritional characteristics and antioxidant activity (Robbins, 2003). In our study, TPC concentrations significantly varied between cultivars evaluated during the harvest periods; in particular, a general decrease or stasis of its content was recorded in all varieties, due to the oxidation of polyphenols by polyphenoloxidase during fruit maturation as well as the biosynthesis of flavylium ring of anthocyanins (Kulkami and Aradhya, 2005; Shwartz et al., 2009). Among all varieties tested,

Wonderful fruits of both provenance showed higher TPC contents (Table 4).
A slight increase was detected with DPPH and FRAP assays both in Italian and Spanish samples during the three harvest periods, but the differences between the cultivars were more evident in ABTS assay. In particular, the highest differences were observed at the first harvest, probably due to the late maturation of the majority of the varieties in observation. Wonderful of Spanish provenance confirm to be earlier in this environment than in the Italian one, probably for the highest average temperatures during maturation recorded in its cultivation area (data not shown).

3.4 Mineral analysis

The minerals content of the pomegranate arils of the considered varieties are shown in Table 6. The data clearly showed that potassium (K) was the predominant macro-element in all cultivars, while zinc (Zn) followed by iron (Fe) were the predominant micro-element in the majority of the cultivars, in according with previous studies (Al-Maiman and Ahmad, 2002; Mirdehghan and Rahemi, 2007). According to Fawole and Opara, 2013b, as maturation progresses there are significant decreases in micro-nutrients (Fe, Zn, Cu, and Mn). Generally, along the maturation period, significant decreases in most of the investigated mineral elements were observed. A different behavior was observed for manganese (Mn) (except for Wonderful of Spanish provenance, probably due to its precocity). A Calcium (Ca) increase during maturation was observed on Valenciana and Mollar de Elche of Italian provenance and on Wonderful of Spanish provenance, whilst a decrease was

observed for the others. This variation among varieties could be attributed to difference in cultivar, plant nutrition, climate and soil conditions (Hamurcu et al., 2010).

Table 1. Mean values of the main morphological parameters of pomegranate fruits at three different harvest time in 2015.

	Weight (g)			D1 (mm)			L1 (mm)		
	17 Sept	**6 Oct**	**21 Oct**	**21 Sept**	**6 Oct**	**21 Oct**	**21 Sept**	**6 Oct**	**21 Oct**
WON-ES	314.2b [a]	303.9c	455.6b	88.7bc	88.8bc	101.7b	78.9b	78.2bc	89.9b
WON-IT	374.1a	461.9a	627.6a	93.2ab	100.1a	109.7a	84.3a	91.1a	100.3a
VAL-ES	413.1a	423.8ab	389.5bc	97.7a	100.2a	96.2bc	80.4ab	80.7b	79.6c
VAL-IT	285.4b	300.4c	341.4c	86.2cd	84.5c	91.2c	73.6c	74.9c	79.2c
MOL-ES	320.3b	411.8ab	453.9b	85.7cd	99.6a	101.8b	71.9cd	80.5b	84.3bc
MOL-IT	268.1b	382.5b	423.6bc	81.6d	92.9b	98.2bc	69.1d	79.3b	81.0c

[a] Values along columns with different letters are different for $P \leq 0.05$

Table 2. Peel colour measured on pomegranate varieties at three different harvest time in 2015.

	17 Sept					6 Oct					21 Oct				
	L*	**a***	**b***	**C**	**h**	**L***	**a***	**b***	**C**	**h**	**L***	**a***	**b***	**C**	**h**
WON-ES	55.2c [b]	37.7a	31.0b	49.7a	41.2b	49.7c	40.7ab	22.8b	47.0a	29.6bc	40.9c	34.7a	11.3e	36.7b	18.0e
WON-IT	61.4b	12.2c	33.6ab	36.6d	69.4a	62.6a	23.2c	29.6a	38.4b	52.3a	53.2b	36.2a	25.8b	44.9a	36.2bc
VAL-ES	51.4c	41.3a	15.3d	44.8ab	21.1c	42.1c	44.7a	16.8c	48.1a	20.7c	37.6c	39.2a	14.8d	41.9ab	20.5de
VAL-IT	62.0b	31.6a	26.4c	42.1bc	40.4b	54.0b	38.4b	24.7b	46.2a	33.5b	50.4b	39.3a	22.5c	45.9a	30.2cd
MOL-ES	68.1a	18.4b	33.9a	40.1c	61.7a	61.3a	25.0c	29.2a	40.5b	50.7a	63.4a	24.6b	30.8a	42.1ab	53.2a
MOL-IT	63.3ab	15.4c	35.3a	39.6d	66.4a	67.3a	18.7c	30.8a	37.0c	58.6a	58.2b	32.0a	30.4a	45.1a	44.5b

[a] Values along columns with different letters are different for $P \leq 0.05$

Table 3. Chemical parameters (total sugars, total acidity and total anthocyanins) measured on pomegranate varieties at three different harvest time in 2015.

	TSS (°Brix)			TA (g L^{-1})			TAC (mg L^{-1})		
	17 Sept	**6 Oct**	**21 Oct**	**21 Sept**	**6 Oct**	**21 Oct**	**21 Sept**	**6 Oct**	**21 Oct**
WON-ES	16.6b [a]	17.4b	18.0a	17.9b	15.8b	13.2b	213.1a	301.9a	403.4a
WON-IT	13.5e	15.3c	16.6b	24.3a	18.2a	15.7a	90.6b	203.9b	334.6b
VAL-ES	17.5a	18.1a	18.1a	2.7c	2.0c	2.0c	75.4b	134.0c	181.2c
VAL-IT	15.9c	15.6c	15.5c	1.9c	1.7c	1.5e	70.4b	79.2d	96.0de
MOL-ES	14.9d	15.8c	15.5c	1.8c	1.9c	1.9cd	29.5c	59.6d	129.4d
MOL-IT	15.6c	15.7c	16.6b	1.8c	1.7c	1.8d	5.8c	56.0d	82.8e

[a] Values along columns with different letters are different for $P \leq 0.05$

Table 4. FRAP, DPPH and ABTS assays (mmol TE kg^{-1} FW) and total polyphenols content (mg GAE L^{-1}) measured on pomegranate varieties at three different harvest time in 2015.

	TPC			FRAP			DPPH			ABTS		
	17 Sept	**6 Oct**	**21 Oct**	**17 Sept**	**6 Oct**	**21 Oct**	**17 Sept**	**6 Oct**	**21 Oct**	**17 Sept**	**6 Oct**	**21 Oct**
WON-ES	486.2a [a]	434.6a	523.3a	47.36a	49.87a	53.7a	18.9b	31.8a	31.62a	21.0a	10.6a	9.9ab
WON-IT	405.0b	471.6a	344.4bc	47.62a	50.22a	47.3ab	32.2a	31.9a	31.69a	8.1b	10.6ab	8.6ab
VAL-ES	413.4b	436.0a	464.6ab	45.45a	50.39a	52.3a	32.4a	32.0a	28.00a	7.2bc	9.9ab	11.2a
VAL-IT	297.9c	272.9c	199.8d	47.36a	47.39a	44.7b	30.5a	30.3b	31.93a	5.1d	5.4c	5.5b
MOL-ES	241.6d	227.0c	285.3cd	45.71a	46.75a	50.6ab	29.3a	31.3ab	31.34a	4.4d	6.6bc	5.6b
MOL-IT	341.1c	316.7b	285.3cd	46.58a	49.09a	51.5a	27.9a	31.5a	31.16a	6.8c	6.8abc	5.8b

[a] Values along columns with different letters are different for $P \leq 0.05$

Table 5. Organic acids and sugars (%) of pomegranate varieties at three different harvest time in 2015.

	IT VAL	ES VAL	IT WON	ES WON	IT MOL	ES MOL
21 September 2015						
ACIDS (%)						
Citric	0.29c	0.22b	0.40d	0.25d	0.26c	0.18a
Malic	0.66a	0.68a	0.24d	0.45bc	0.40c	0.51b
Quinic	1.26b	0.82c	1.97a	0e	0.92c	0.50d
SUGARS (%)						
Glucose	5.48b	5.46b	3.82d	5.41b	4.79c	10.76a
Fructose	9.40b	9.44b	5.62d	12.94a	8.30c	9.12b
6 October 2015						
ACIDS (%)						
Citric	n.d.	0.25b	2.55a	2.41a	0.20b	0.19b
Malic	n.d.	0.91a	0.42c	0.61b	0.44c	0.55b
Quinic	n.d.	1.14c	1.83b	2.15a	0.71d	0.76d
SUGARS (%)						
Glucose	n.d.	6.48a	3.57c	5.73ab	4.81bc	5.70ab
Fructose	n.d.	11.16a	6.72d	9.70b	8.65c	9.59b
21 October 2015						
ACIDS (%)						
Citric	0.12e	0.35c	2.32a	2.23b	0.25d	0.19de
Malic	0.51c	0.81a	0.50c	0.65b	0.52c	0.48c
Quinic	0.83d	1.00c	1.43b	1.81a	0.78d	0.57e
SUGARS (%)						
Glucose	4.72b	6.04a	4.25b	6.19a	5.15ab	5.10ab
Fructose	8.92b	10.47a	9.07b	9.42b	8.95b	9.04b

[a] Values along rows with different letters are different for $P \leq 0.05$

Table 6. Mineral content (macro and micro-elements expressed as g kg^{-1} DW and mg kg^{-1} DW, respectively) of pomegranate varieties at three different harvest time in 2015.

	21 September					
	IT VAL	**ES VAL**	**IT WON**	**ES WON**	**IT MOL**	**ES MOL**
Macro-elements						
Calcium (Ca)	36.9bcd [a]	42.9abc	46.2ab	27.4cd	25.0d	53.2a
Magnesium (Mg)	261.2c	196.1d	473.0b	740.0a	255.1c	253.2c
Potassium (K)	2814.0b	2692.9bc	3706.7a	2528.4c	2959.7b	2755.1bc
Micro-elements						
Iron (Fe)	16.1ab	12.4b	24.9ab	33.7a	20.8ab	9.4b
Zinc (Zn)	24.0c	16.1e	35.3a	21.3d	26.8b	15.0e
Copper (Cu)	21.0c	14.7d	29.6a	13.7d	24.0b	11.1e
Manganese (Mn)	10.1e	13.0d	23.3a	15.5b	14.3c	14.7bc
	6 October					
Macro-elements						
Calcium (Ca)	40.0ab	20.1c	51.9a	41.7ab	53.7a	29.4bc
Magnesium (Mg)	315.6c	465.6b	321.6c	634.2a	215.4d	534.3b
Potassium (K)	2867.0a	2658.1a	2954.0a	2683.8a	2832.1a	3119.7a
Micro-elements						
Iron (Fe)	17.4b	4.9d	25.0a	13.2c	18.2b	2.9d
Zinc (Zn)	20.7ab	16.4b	26.0a	24.3a	23.6a	21.4ab
Copper (Cu)	19.7b	13.0d	22.5a	16.7c	20.2b	9.4e
Manganese (Mn)	9.5d	8.4d	22.4a	18.9b	16.4c	9.8d
	21 October 2015					
Macro-elements						
Calcium (Ca)	41.3a [a]	31.2ab	39.7a	42.1a	133.8b	28.7ab
Magnesium (Mg)	398.6c	460.3b	512.2ab	506.6ab	535.3a	544.2a
Potassium (K)	2396.8b	2513.1ab	2586.4ab	2852.7ab	2878.9ab	3216.9a
Micro-elements						
Iron (Fe)	14.4ab	7.5c	15.9a	8.3c	10.0bc	4.6c
Zinc (Zn)	18.6b	13.1c	30.2a	17.4b	30.1a	14.4bc
Copper (Cu)	18.0b	9.4d	20.5a	13.5c	17.9b	7.8e
Manganese (Mn)	8.2c	8.5c	15.6a	8.1c	8.1c	12.6b

[a] Values along rows with different letters are different for $P \leq 0.05$

4. Conclusions

Pomegranate represent a species with a rather poor number of contributions regarding several aspects of its agronomy, cultivar selection, postharvest management. Nevertheless in the past few years remarkable work has been done in this direction and the evidence of some cultivar more appreciated in the market for their attractiveness is now evident. However little is known about the behavior of these cultivar in different cultivation areas. In this work a comparison of some important genotypes cultivated in two different Mediterranean areas of Spain and of Italy has been carried out. A number of pomological and qualitative parameters have been measured also considering a rather wide maturation calendar. It is known that environmental conditions strongly affect several quality parameters as colour, taste (TSS:TA ratio) and nutraceutical compounds.

The results of this work confirm some already achieved evidences on the fruit characteristic of Wonderful a variety with peculiar organoleptic traits and with a deep and uniform red colour of the fruits. The acidity levels of Wonderful resulted to be about ten fold higher than those of the other tested varieties independently from the cultivation area, confirming a very different qualitative profile of this cultivar that should be taken into consideration when varietal choices are made at least for fruits to be sold as fresh.

As for the influence of the cultivation area the results herein achieved testify as the peculiar climatic conditions of each are may contribute to improve some qualitative aspects of selected genotypes. This is particularly true for the aspects

related to fruit colour and especially to its changes along maturation. The Spanish environment taken into consideration in this study seems to be more able to fasten ripening process in at least two of the tested varieties; also higher values of both TSS and TA were recorded in fruits of Wonderful and Valenciana of Spanish provenance.

Experimental study # 4

Anthocyanin characterization and antioxidant capacity of some Sicilian pomegranate (*Punica granatum* L.) accessions in comparison with international varieties

1. Introduction

Nowadays, one of the most important parameter to which consumers are sensitive when selecting fruits and vegetables (i.e. red orange, pomegranate, grape, berries, tomato, etc.) is the colour. In particular, red colour, together with blue, are considered of great importance in fruit and vegetable because of their benefit for the human health, as they contain several substances helpful for disease prevention. Commonly, the red colour is associate by the presence of anthocyanins, natural antioxidants (Navindra et al., 2006). Pomegranate (*P. granatum* L.) is a rich source of bioactive compounds useful for disease prevention; the anthocyanins identified in fruits are six: delphinidin 3- and 3,5-diglucoside, cyanidin 3- and 3,5-diglucoside, pelargonidin 3- and 3,5-diglucoside (Gil et al., 1995a).
Because of the increasingly market demand of these natural functional products, it is important to characterize among the vast germplasm local pomegranate accessions with high quality parameters. The aim of this work was to investigate the evolution of quality parameters of Sicilian accessions in comparison with the worldwide commercial cultivars during three harvest times, in order to evaluate the maturation

evolution and their adaptability for fresh and/or industrial processing demand.

2. Material and methods

2.1 Plant material

Five Sicilian local accessions, namely Dente di Cavallo (DDC), Primosole (PRIM), PG-CT5 (PG-5), PG-CT6 (PG-6), Valenti (VAL), and four commercial worldwide cultivars, Wonderful (WON), Akko (AKK), Parnipal (PAR,) Mollar de Elche (MOL), were grown applying standard horticultural practices in the experimental farm of the Catania University (Italy) located near the eastern coast of Sicily (37°24'37'' N; 15°03'16'' E). The collection field was constituted of four trees for each variety. Pomegranate fruits were collected from the four tree sides at mid-height (4 fruit per tree side) at three harvest times: fruits were picked weekly (8, 15 and 22 of October) during 2014 and every 15 days (17 of September, 5 and 20 of October) during 2015. Fruits were transported to the laboratory, and used for physical-chemical determinations.

2.2 Quality parameters determination

Pomological and chemical parameters as peel and juice colour, total solid soluble (TSS), titratable acidity (TA), vitamin C (L-ascorbic acid) were measured as reported in section 2.3 "Experimental study #1", while total polyphenols content (TPC) and ORAC-value were measured as reported in section 2.5 of "Experimental study #1".

2.3 HPLC/DAD and HPLC/ESI/MS anthocyanin analysis

Anthocyanin content was quantify using HPLC-DAD method as reported in section 2.4 “Experimental study #1”.

2.4 Statistical analysis

The statistical analysis was carried out as reported in section 2.6 of “Experimental study #1”.

3. Results and discussion

3.1 Colour and chemical analyses

Pomegranate acceptability by consumers and processors depends basically on a combination of several quality attributes as rind colour, sugar content, acidity, and flavour (Al-Said et al., 2009; Viuda-Martos et al., 2010). Peel and juice colour are considered important quality attributes in pomegranate marketing because the reddish-purple colouration is commonly associated with healthy benefit (Seeram et al., 2006).
During the two years of this study, the peel colour have shown a common increase of a* (redness) and a decrease of b* values for all accessions evaluated (Table 1 and 2), due to the evolution of pomegranate fruit maturation. The b* values of pomegranate rind significantly fell from the second week of October onward, indicating that blue pigments were replacing the yellow colour during fruit maturation. In 2015 Dente di Cavallo, PG-CT5 and Primosole accessions showed a negative a* value at first

harvest time in September and they reached positive values only in October (Table 2) where the green peel colour was increasingly replaced by the red one. Lower values in 2015 than in 2014 were recorded in all accessions and cultivars in evaluation: this was probably due to the higher summer temperatures during 2015 (data not shown) that delay the pigmentation of the peel, as confirmed in previous works (Al-Maiman et al., 2002; Gil et al., 1995a; Manera et al., 2011; Shwartz et al., 2009). Peel colour recorded on all local accessions did not differ so much with the colour observed in international varieties.

As regard the juice colour, during the two years no great differences were recorded by Sicilian accessions PG-CT5, PG-CT6, Primosole, while a slight change of colour (reddish-purple) is observed on Dente di Cavallo along the three harvest times evaluated. Differently, for the medium-late varieties Wonderful and Akko, a great decrease of a* and increase of hue angle us understand the change colour of juice from the second week of October onward (Tables 3 and 4).

Table 1. Peel colour measured on pomegranate samples at three harvest times in 2014.

	PEEL COLOUR														
	8 October					15 October					22 October				
	L*	a*	b*	C	h	L*	a*	b*	C	h	L*	a*	b*	C	h
AKK	52.5±6.3[a]	32.6±11.2	32.6±3.5	47.2±5.1	46.3±13.6	52.4±11.2	43.1±7.7	26.5±6.5	51.1±6.7	31.6±9.1	50,6±7.5	38.7±8.0	28.0±2.9	48.1±5.7	36.6±7.9
WON	49.2±5.9	38.1±8.8	29.9±4.3	49.2±4.4	38.9±10.6	45±4.5	48.8±4.8	21.5±2.8	53.1±4.8	24.0±3.2	55.9±7.1	23.2±10.1	31.6±3.9	40.2±6.0	54.8±12.6
MOL	56.8±7.2	35.3±11.5	31.7±3.9	48.6±4.8	43.2±14.0	46.5±6.7	30.3±11.4	29.7±3.8	43.4±7.6	46.1±12.4	46.6±4.5	39.3±3.7	27.4±4.5	48.1±2.6	34.9±6.1
DDC	61.8±10.6	9.3±10.3	33.4±7.9	36.3±6.6	72.7±18.3	67.7±6.5	11.4±15.4	35.3±5.1	40.0±4.2	72.3±22.1	71.4±5.5	2.7±6.8	38.0±4.2	38.7±3.3	85.2±11.0
PAR	51.8±7.3	42.0±10.4	28.8±3.9	51.8±5.0	35.5±12.2	60.7±7.0	34.7±10.4	28.05±2.8	45.4±5.7	40.5±12.7	48.4±5.3	44.6±5.5	25.7±4.2	51.7±4.1	30.2±6.4
PG-5	58.3±8.0	20.4±11.0	32.6±5.4	40.0±3.9	58.4±17.0	50.8±4.7	38.5±7.2	27.0±3.1	47.4±4.8	35.6±7.9	47.9±5.0	41.1±5.6	24.8±3.7	48.2±4.0	31.4±6.7
PG-6	52.1±7.0	34.2±10.2	27.6±3.9	45.3±5.9	39.6±12.4	51.1±9.4	32.0±9.7	28.7±3.6	43.7±6.2	43.0±11.6	47.2±2.4	44.6±4.7	26.2±3.6	52.0±3.1	30.6±5.6
PRIM	52.5±7.9	30.8±12.7	29.4±5.2	44.3±5.1	45.5±17.3	49.9±7.9	33.0±16.0	27.6±4.8	44.9±9.7	42.9±18.4	46.2±6.7	36.0±11.2	28.2±4.1	43.2±8.2	40.7±13.2
VAL	53.6±6.8	37.7±9.9	29.7±4.4	48.8±5.3	39.2±12.0	53.3±5.2	37.2±11.9	28.6±4.7	48.1±6.6	39.1±14.7	51.5±8.3	36.5±9.7	29.0±3.6	47.3±6.3	39.6±10.4

[a] Mean ± st.dv (Standard deviation)

Table 2. Peel colour measured on pomegranate samples at three harvest times in 2015.

	PEEL COLOUR														
	17 September					05 October					20 October				
	L*	a*	b*	C	h	L*	a*	b*	C	h	L*	a*	b*	C	h
AKK	62.3±7.4	1.64±3.7	39.5±2.2	39.1±3.3	87.6±5.4	60.7±9.6	20.0±15.2	36.2±4.1	43.8±5.4	63.0±19.4	59.9±10.6	31.2±10.3	29.1±2.9	43.8±7.0	45.7±12.3
WON	61.0±3.9	7.4±4.8	37.8±3.1	38.8±3.1	78.9±7.1	60.7±6.3	21.5±7.3	32.6±3.5	39.8±3.0	56.9±10.9	55.4±4.1	35.8±8.2	27.6±2.0	45.5±6.4	38.5±7.6
MOL	60.7±7.4	8.2±7.2	38.2±3.5	39.7±3.2	77.7±10.6	66.6±8.7	16.3±16.2	37.4±4.7	43.8±5.3	67.9±21.1	59.6±9.3	30.3±12.3	32.1±2.8	45.4±6.6	48.6±14.5
DDC	62.5±7.7	-2.4±14.7	37.0±6.0	39.8±5.0	91.7±23.1	65.0±6.1	-0.8±17.2	38.9±5.8	42.6±3.4	89.2±25.3	65.0±5.1	4.1±10.5	36.4±3.9	38.0±3.8	83.0±15.6
PAR	69.8±5.7	11.3±7.2	39.7±1.8	41.9±1.4	74.3±9.9	66.6±9.2	24.5±14.0	34.1±3.6	43.9±6.2	56.3±17.4	55.7±9.1	41.3±9.0	28.28±2.2	50.8±6.1	35.8±8.3
PG-5	55.68.5±	-1.3±14.2	36.3±6.7	39.2±4.6	91.2±22.6	63.0±8.5	18.5±13.4	37.5±2.9	43.7±3.9	65.1±17.5	60.6±8.9	28.4±13.7	31.5±3.9	44.2±5.9	50.0±17.5
PG-6	58.5±7.5	1.9±13.3	37.8±4.4	40.1±3.2	85.9±19.9	61.1±11.0	17.6±14.8	34.1±6.3	41.1±5.6	63.5±21.2	59.1±10.5	31.715.4	31.0±4.7	46.34±6.7	46.6±19.0
PRIM	58.5±9.3	-3.8±11.1	37.2±7.7	39.1±6.6	92.6±20.2	62.8±8.3	11.0±15.1	38.3±4.0	42.5±3.6	74.6±20.6	60.2±9.9	26.8±15.2	32.9±5.0	44.8±6.1	53.2±19.7
VAL	60.0±10.0	11.7±8.6	37.9±7.0	40.8±5.7	72.3±14.3	60.9±10.4	29.0±13.2	32.4±3.7	45.1±6.5	50.3±15.8	53.2±7.8	36.3±10.8	29.8±2.9	47.8±5.8	40.8±12.6

[a] Mean ± st.dv (Standard deviation)

Table 3. Juice colour measured on pomegranate samples at three harvest times in 2014.

	JUICE COLOUR														
	8 October					15 October					22 October				
	L*	a*	b*	C	h	L*	a*	b*	C	h	L*	a*	b*	C	h
AKK	18.4±0.03[a]	2.4±0.1	1.5±0.01	2.9±0.1	31.4±1.21	18.2±0,01	1.8±0.1	1.2±0.1	2.1±0.01	33.2±3.0	18.1±0.04	1.6±0.1	1.1±0.1	1.9±0.1	33.2±1.2
WON	18.5±0.01	3.0±0.01	1.8±0.03	3.5±0.02	30.1±0.5	18.9±0.7	2.7±0.2	1.5±0.03	3.1±0.2	29.5±1.3	18.3±0.02	1.9±0.1	1.2±0.1	2.2±0.01	32.8±3.1
MOL	20.2±0.03	4.1±0.1	1.8±0.04	4.4±0.1	24.5±0.9	19.5±0.01	4.4±0.1	2.0±0.1	4.8±0.1	24.3±0.2	21.2±0.2	3.3±0.04	1.5±0.04	3.6±0.05	25.0±0.2
DDC	20.0±0.01	4.5±0.1	2.3±0.03	5.1±0.1	27.4±0.7	18.9±0.1	3.5±0.04	2.1±0.04	4.1±0.02	31.1±0.8	18.6±0.02	3.3±0.1	1.9±0.04	3.8±0.05	29.1±0.1
PAR	22.1±0.01	2.3±0.1	1.6±0.03	2.8±0.05	34.6±1.1	20.9±0.01	3.6±0.01	2.1±0.01	4.2±0.01	30.4±0.01	22.4±0.02	1.7±0.01	1.4±0.01	2.2±0.01	39.8±0.2
PG-5	19.8±0.02	4.2±0.1	1.7±0.1	3.9±1.2	15.3±12.4	19.2±0.04	4.2±0.1	1.9±0.02	4.6±0.09	24.7±0.8	19.1±0.02	4.2±0.2	1.6±0.01	4.6±0.2	21.2±0.8
PG-6	20.1±0.02	4.2±0.1	1.8±0.05	4.6±0.1	22.9±0.9	19.6±0.01	4.3±0.03	1.5±0.1	4.6±0.04	19.5±0.6	19.5±0.02	4.2±0.06	1.8±0.04	4.6±0.1	23.5±0.2
PRIM	19.9±0.03	4.0±0.02	2.2±0.05	4.5±0.02	29.3±0.6	19.6±0.2	4.5±0.3	2.1±0.1	5.0±0.3	24.7±0.3	19.62±0.1	4.1±0.2	1.7±0.1	4.6±0.3	21.4±0.2
VAL	19.1±0.04	3.9±0.1	1.9±0.1	4.3±0.2	25.5±0.8	20.2±0.1	4.3±0.02	1.6±0.1	4.6±0.1	20.2±1.0	19.5±0.01	4.4±0.2	1.7±0.01	4.8±0.2	21.3±0.9

[a] Mean ± st.dv (Standard deviation)

Table 4. Juice colour measured on pomegranate samples at three harvest times in 2015.

	JUICE COLOUR														
	17 September					05 October					20 October				
	L*	a*	b*	C	h	L*	a*	b*	C	h	L*	a*	b*	C	h
AKK	19.0±0.04	3.3±0.01	2.8±0.1	4.4±0.1	40.5±1.4	18.9±0.6	2.3±0.1	1.9±0.1	3.0±0.01	39.4±1.5	18.1±0.1	1.5±0.2	1.6±0.1	2.2±0.2	46.8±2.4
WON	19.4±0.3	3.7±0.1	2.6±0.3	4.5±0.2	34.1±2.1	18.7±0.4	2.2±0.1	2.0±0.03	3.0±0.1	42.6±1.0	17.4±0.7	1.9±0.2	1.9±0.2	2.7±0.3	45.4±1.0
MOL	22.0±0.05	0.7±0.2	3.4±0.2	3.5±0.1	78.4±4.7	21.2±0.6	2.7±0.3	2.51±0.5	3.7±0.1	42.5±8.1	19.5±0.1	4.0±0.2	2.7±0.1	4.9±0.1	33.8±1.8
DDC	19.6±0.01	3.9±0.1	3.3±0.05	5.1±0.1	40.1±0.3	18.5±0.3	3.4±0.1	2.8±0.13	4.4±0.2	38.9±1.0	20.1±0.02	2.9±0.1	3.1±0.03	4.2±0.04	46.2±1.0
PAR	21.9±0.8	1.6±1.5	3.1±0.7	3.8±0.2	64.5±25.6	19.1±2.1	2.0±1.9	1.9±0.5	2.9±1.7	54.4±25.2	19.8±1.5	3.4±0.7	2.3±0.8	4.1±1.0	33.6±3.8
PG-5	21.1±0.04	3.2±0.1	2.7±0.02	4.2±0.1	40.1±0.5	20.4±0.01	3.5±0.04	2.8±0.01	4.5±0.02	39.0±0.3	18.9±0.3	3.8±0.1	2.5±0.1	4.5±.0.2	33.3±0.2
PG-6	21.4±0.01	1.7±0.01	2.3±0.1	2.9±0.1	53.6±1.2	20.1±0.01	3.7±0.01	2.7±0.01	4.6±0.01	35.6±0.2	18.9±0.3	3.9±0.1	2.5±0.2	4.6±0.2	33.0±1.2
PRIM	21.1±0.1	2.8±0.2	2.9±0.3	4.1±0.1	46.6±4.6	19.8±0.04	3.9±0.04	2.6±0.2	4.7±0.1	33.4±2.0	18.4±0.7	3.8±0.4	2.8±0.4	4.7±0.5	35.5±2.6
VAL	22.9±0.1	0.1±0.1	3.9±0.1	3.9±0.1	88.7±1.0	19.8±0.04	3.8±0.2	2.8±0.04	4.7±0.2	35.7±0.9	20.1±0.3	3.5±0.2	2.3±0.1	4.2±0.1	33.8±2.8

TSS of mature pomegranate juice ranging from 12-16 °Brix. Titratable acidity generally decreases with advancing fruit maturation but the % of decline is strongly correlated with cultivars and growing regions; the ascorbic acid concentration normally decrease during the initial stages of fruit maturation (Fawole and Opara, 2013a - 2013b).

The values of total soluble solids (TSS), titratable acidity (TA) and ascorbic acid (Figure 1, 2 and 3, respectively) shown similar trend during the two years tested, while a strange increase of total acidity was shown by Akko variety from the second week of October onward, coinciding with the increase of ascorbic acid at the third week of October (Figure 3). An increase of ascorbic acid concentration are shown by Wonderful during the third week of 2015, with a similar trend reported by Shwartz et al. (2009). Primosole, followed by Dente di Cavallo, have shown an interesting higher content of vitamin C at the first week of October 2014, data not confirmed during 2015 (Figure 3).

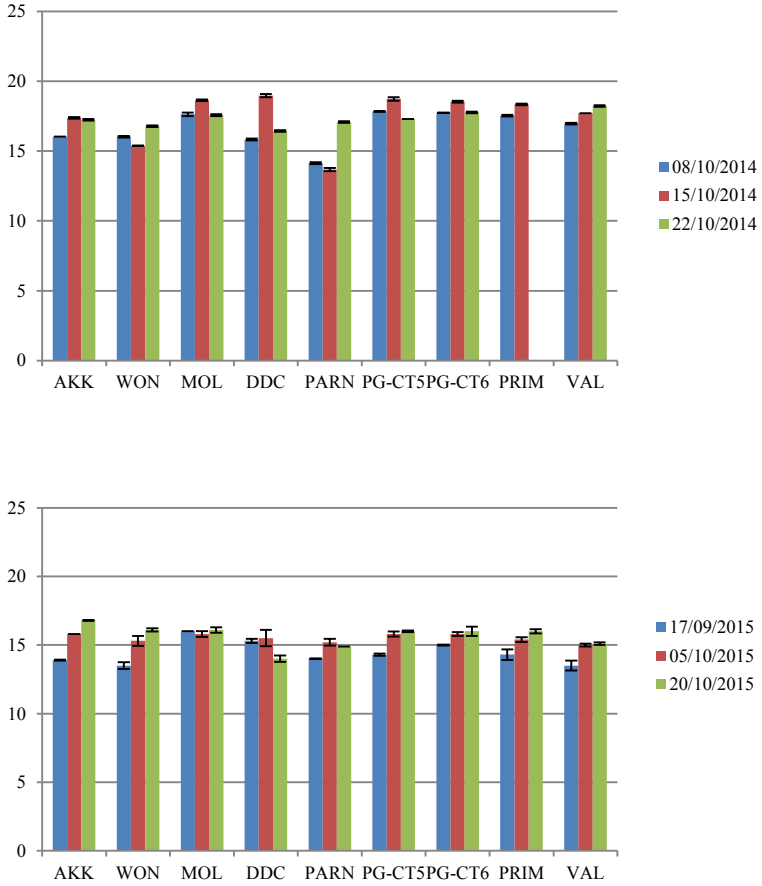


Figure 1. Total soluble solids (°Brix) of pomegranate varieties at three harvest times in 2014 (above) and 2015 (below).

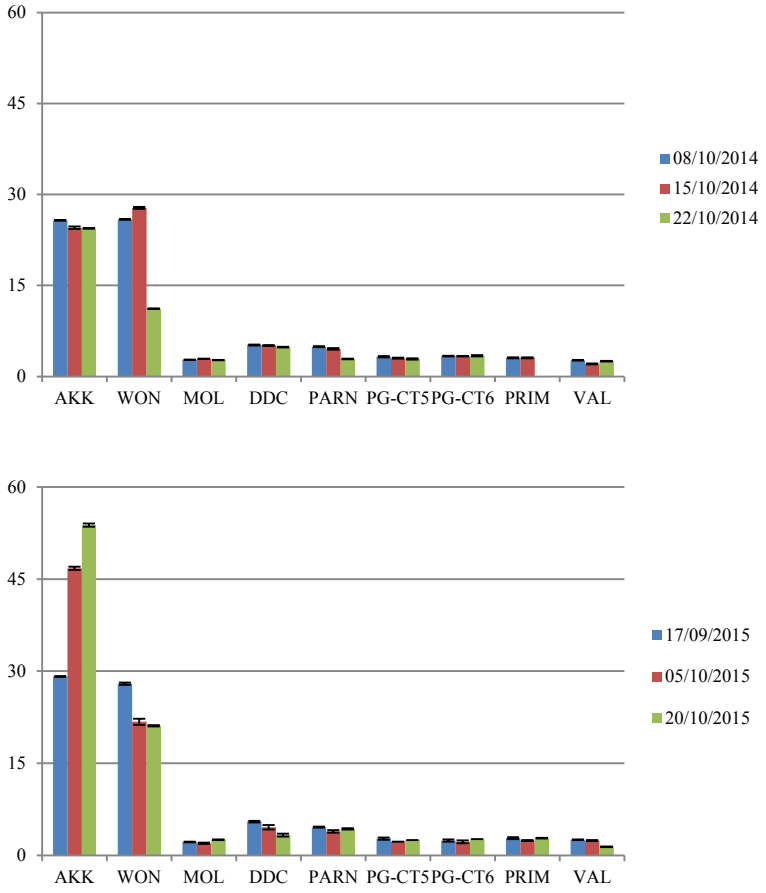


Figure 2. Titratable acidity (g L^{-1}) of pomegranate varieties at three harvest times in 2014 (above) and 2015 (below).

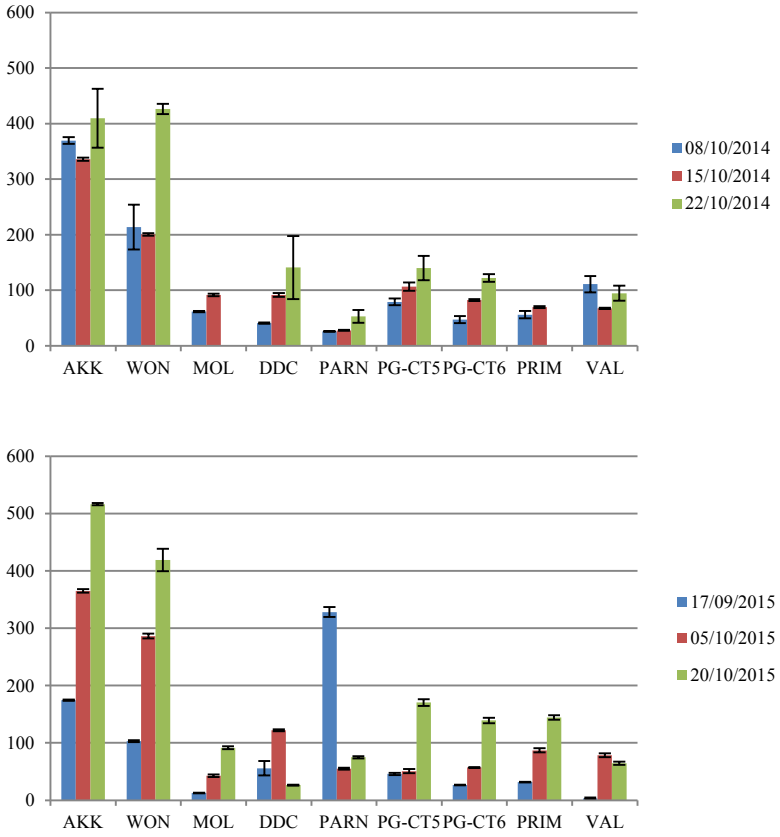


Figure 3. Ascorbic acid (mg L^{-1}) of pomegranate varieties at three harvest times in 2014 (above) and 2015 (below).

3.2. HPLC/DAD and HPLC/ESI/MS anthocyanin analysis

The anthocyanin profile of pomegranate juice is known to be constituted of six anthocyanins: delphinidin-3,5-diglucoside, cyanidin-3,5-diglucoside, delphinidin-3-glucoside, pelargonidin-3,5-diglucoside, cyanidin-3-glucoside and pelargonidin-3-glucoside (Fawole and Opara, 2013b; Türkyılmaz, 2013). Generally, there is an increase in juice pigmentation with fruit ripening. Delphinidin 3,5-diglucoside was identified as the dominant pigment in early ripening stages while, the monoglucoside derivatives of cyanidin 3-glucoside and delphinidin 3-glucoside increased considerably in the later stages (Gil et al., 1995a; Fawole and Opara, 2013b). However several study showed the same anthocyanin profile in all the cultivars, but the total amount of anthocyanins was largely affected by differences in cultivar, maturation stage and the geographical source of the fruit (Gil et al., 1995a).

Total anthocyanin content values revealed a high variability among the accessions and varieties; in fact, the sweet-sour Akko and Wonderful varieties showed the highest anthocyanin content during three harvest times in 2014 and 2015, up to more than 500 mg L^{-1} compared to sweet pomegranate accessions (Figure 5). Interesting increase of anthocyanin content values are shown by the Sicilian accession PG-CT5, PG-CT6 and Primosole both in 2014 and 2015; these values are interestingly coupled with lower acidity contents (Figure 2) and mostly in accordance to those reported in literature (Gómez-Caravaca et al., 2013; Gil et al., 2000; Fawole and Opara, 2013b; Fischer et al., 2011).

In this study during the first harvest period in 2015 the predominant anthocyanin was found to be delphinidin 3,5-diglucoside for the sweet-sour Akko and Wonderful varieties, and cyanidin 3,5-glucoside for the sweetest ones, while the monoglucoside derivatives of cyanidin 3-glucoside and delphinidin 3-glucoside increase in the later stages, i.e. at the end of October (Table 6).

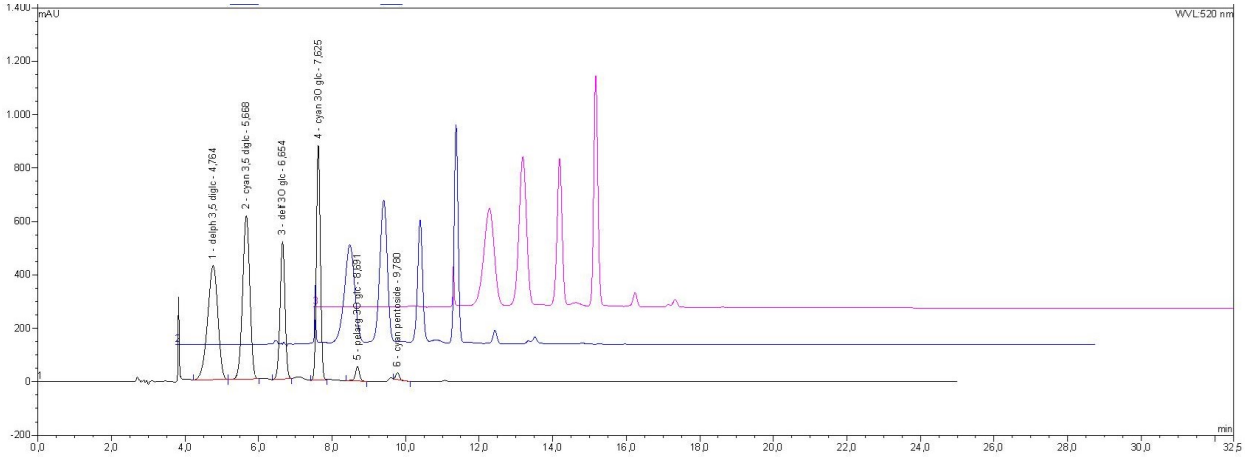


Figure 4. HPLC chromatogram visualized at 520 nm of pomegranate juice for anthocyanins content (SLT = T0). Peak letters and numbers refer to text and are listed in Table 5.

Table 5. Peak list and diagnostics of nine pomegranate juices for anthocyanins content. Peak letters refer to Figure 3.

	Compound identification [a]	**MW**	**Rt**
A1	delphinidin 3,5 diglucoside	627,52	4,076
A2	cyanidin 3,5 diglucoside	611,52	4,931
A3	delphinidin 3-O-glucoside	465,38	5,919
A4	cyanidin 3-O-glucoside	449,38	6,848
A5	pelargonidin 3-O-glucoside	433,38	7,838
A6	cyanidin pentoside	419,24	8,937

[a] co-injection with pure analytical standards;

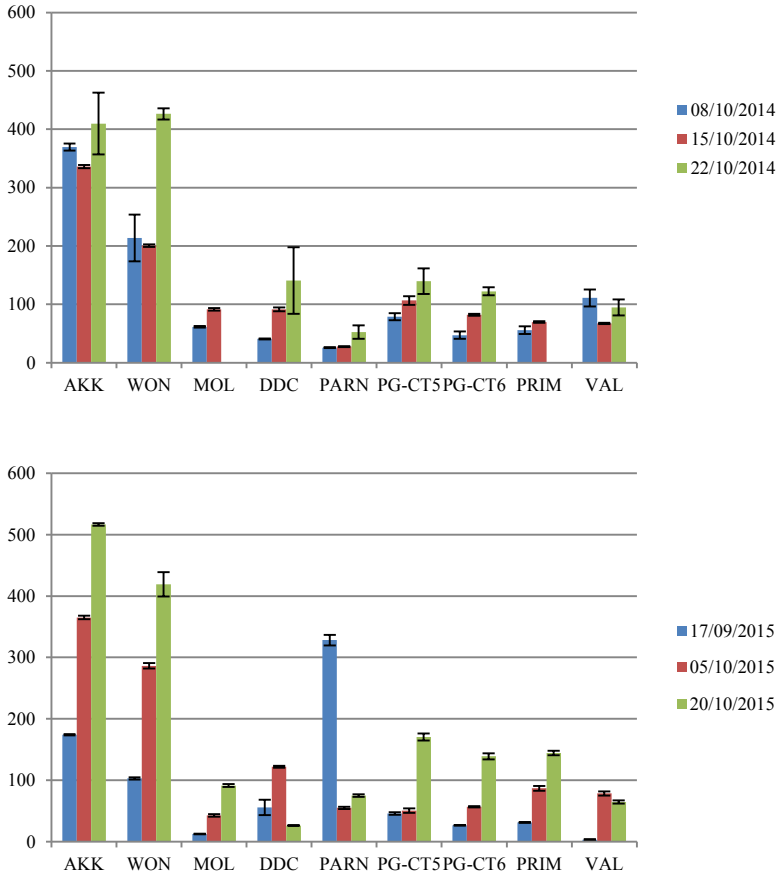


Figure 5. Total anthocyanin content (TAC) measured by HPLC on pomegranate varieties at three harvest times in 2014 (above) and 2015 (below).

Table 6. Individual anthocyanin content of pomegranate varieties at three harvest times in 2014 (above) and 2015 (below). Peak letters (A1-A6) refer to Table 5.

	AKKO			WOND			MOLLAR		
	08 Oct	15 Oct	22 Oct	08 Oct	15 Oct	22 Oct	08 Oct	15 Oct	22 Oct
A1	122.7±1.9	108.7±2.5	125.6±4.9	74.0±15.5	73.2±0.8	138.8±2.2	5.9±0.1	15.4±0.7	n.d.
A2	120.0±1.9	109.0±1.3	119.4±4.9	74.0±12.8	53.3±1.5	118.0±13.5	25.0±0.5	29.9±0.5	n.d.
A3	52.1±0.9	48.5±1.1	71.8±13.8	30.0±5.1	38.0±0.6	84.0±6.9	3.0±0.01	9.6±0.9	n.d.
A4	70.4±1.5	65.7±0.4	87.7±13.7	34.9±6.7	33.1±0.9	79.8±1.7	25.5±0.7	32.3±1.3	n.d.
A5	4.3±0.1	4.0±0.1	5.2±0.9	2.5±0.4	2.7±0.05	5.7±0.2	2.0±0.03	4.2±0.1	n.d.
A6	1.6±0.1	1.3±0.02	2.3±0.4	1.0±0.2	1.2±0.05	4.0±0.9	0.3±0.02	0.4±0.01	n.d.
	DDC			**PARNIPAL**			**PG-CT5**		
	08 Oct	15 Oct	22 Oct	08 Oct	15 Oct	22 Oct	08 Oct	15 Oct	22 Oct
A1	3.2±0.2	13.0±0.7	10.6±7.0	2.3±0.2	2.6±0.2	10.5±1.0	13.3±2.9	28.2±1.1	31.8±10.4
A2	17.2±0.3	23.0±0.8	24.4±3.8	14.9±0.8	13.1±0.6	29.6±7.8	33.9±0.9	30.1±4.7	44.3±6.0
A3	2.5±0.2	14.3±0.6	35.7±25.0	1.1±0.04	1.7±0.1	3.5±0.6	5.8±1.2	18.0±0.6	23.1±12.1
A4	16.9±0.5	37.3±1.7	64.5±21.8	6.9±0.03	9.4±0.3	7.9±2.0	24.7±1.0	28.2±1.7	38.6±5.3
A5	1.0±0.03	3.9±0.1	5.6±1.9	0.5±0.01	0.8±0.02	1.2±0.3	1.2±0.03	2.0±0.15	2.1±0.3
A6	0.4±0.01	0.8±0.02	1.0±0.3	0.1±0.02	0.2±0.00	0.1±0.1	0.3±0.4	0.9±0.4	1.8±1.1
	PG-CT6			**PRIMOSOLE**			**VALENTI**		
	08 Oct	15 Oct	22 Oct	08 Oct	15 Oct	22 Oct	08 Oct	15 Oct	22 Oct
A1	5.1±0.4	19.3±0.6	16.8±4.5	9.7±0.4	18.9±0.8	n.d.	20.6±0.9	14.3±0.9	15.0±8.9
A2	18.6±2.8	16.3±1.6	34.1±9.9	26.8±4.0	23.3±0.4	n.d.	44.8±7.8	27.1±0.3	35.4±7.7
A3	3.0±0.3	20.0±1.1	19.8±9.9	3.5±0.2	10.4±0.8	n.d.	8.9±0.7	5.5±0.2	12.5±7.9
A4	19.5±2.8	25.2±0.6	48.6±2.1	15.2±2.0	16.4±0.5	n.d.	34.9±5.1	18.9±1.0	29.9±5.1
A5	0.9±0.1	1.2±0.01	3.1±0.3	0.7±0.1	0.9±0.1	n.d.	1.6±0.3	1.3±0.02	1.8±0.3
A6	0.3±0.05	0.9±0.5	2.0±1.1	0.2±0.03	0.3±0.02	n.d.	0.4±0.1	0.2±0.02	0.2±0.1

continuing

	AKKO			WOND			MOLLAR		
	17 Sept	05 Oct	20 Oct	17 Sept	05 Oct	20 Oct	17 Sept	05 Oct	20 Oct
A1	70.1±1.8	116.8±3.4	136.2±1.6	40.9±0.5	86.6±1.0	16.1±0.1	1.4±0.03	1.7±0.3	5.0±0.04
A2	70.0±1.0	139.8±1.5	172.1±1.3	39.2±1.2	96.1±1.9	77.3±1.5	8.0±0.6	21.2±0.7	46.3±1.3
A3	13.9±0.04	36.2±2.0	73.8±0.9	10.2±0.8	39.2±1.0	10.0±0.6	0.3±0.01	1.1±0.1	3.9±0.1
A4	18.6±0.1	65.6±0.1	121.7±1.3	11.7±0.1	58.4±1.4	39.5±1.9	2.6±0.03	16.8±0.9	31.7±0.8
A5	1.3±0.1	4.3±0.1	8.1±0.1	0.9±0.03	3.5±0.1	6.1±0.2	0.2±0.1	1.7±0.1	4.1±0.3
A6	0.4±0.03	2.4±0.1	4.5±0.1	0.3±0.01	2.5±0.2	0.5±0.1	0.04±0.0	0.3±0.01	0.5±0.1
	DDC			**PARNIPAL**			**PG-CT5**		
	17 Sept	05 Oct	20 Oct	17 Sept	05 Oct	20 Oct	17 Sept	05 Oct	20 Oct
A1	9.6±1.8	11.1±1.2	0.8±0.03	0.4±0.1	6.3±0.4	10.6±0.6	n.d.	2.1±0.4	22.2±1.0
A2	25.4±3.1	49.0±0.1	9.4±0.2	7.2±0.5	28.9±0.6	34.9±0.6	n.d.	27.0±1.7	64.4±2.0
A3	5.7±2.2	8.4±0.4	1.1±0.2	0.2±0.1	2.5±0.2	5.0±0.3	n.d.	1.3±0.2	12.9±1.2
A4	13.8±8.6	48.6±1.3	13.9±0.5	1.4±0.1	15.4±0.6	21.3±0.5	n.d.	18.7±1.4	63.9±1.4
A5	1.0±0.6	3.4±0.02	0.9±0.02	0.1±0.0	1.8±0.1	2.9±0.3	n.d.	1.3±0.1	5.9±0.3
A6	0.3±0.1	1.6±0.03	0.4±0.01	0.1±0.03	0.3±0.03	0.3±0.01	n.d.	0.4±0.04	1.1±0.02
	PG-CT6			**PRIMOSOLE**			**VALENTI**		
	17 Sept	05 Oct	20 Oct	17 Sept	05 Oct	20 Oct	17 Sept	05 Oct	20 Oct
A1	2.9±0.5	3.0±0.3	17.1±1.3	6.0±0.2	14.7±0.8	17.6±1.4	0.04±0.0	4.5±0.3	3.1±0.3
A2	15.2±0.7	24.0±0.5	54.7±2.3	17.0±0.6	34.1±1.2	54.4±1.7	2.6±1.2	33.8±2.0	32.2±1.4
A3	1.1±0.2	2.1±0.2	9.6±0.5	1.9±0.1	6.8±0.3	11.9±2.0	0.02±0	3.3±0.3	1.9±0.1
A4	7.0±0.1	25.9±1.3	51.8±1.6	6.2±0.1	29.0±1.5	54.5±1.0	0.6±0.1	34.1±1.1	24.8±1.0
A5	0.3±0.1	1.6±0.1	4.8±0.1	0.2±0.04	1.9±0.1	4.8±0.1	0.01±0	2.4±0.1	2.1±0.2
A6	0.1±0.02	0.4±0.1	1.0±0.01	0.1±0.01	0.5±0.1	1.1±0.1	0.03±0	0.4±0.1	0.4±0.1

[a] Mean ± st.dv (Standard deviation)

3.3 Antioxidant activity (ORAC, ABTS+ and DPPH• methods) and total polyphenols

Total polyphenols content (TPC) values significantly varied among the accessions and varieties evaluated (Figure 6). Among these, during the two years, and according to the considered sampling date, the content of TPC is comparable. Higher values of TPC (~2475 mg GAE L^{-1}) were found in Wonderful, at the occasion of the harvest occurred in mid Seeptember; interestingly at that time, also Primosole, PG-CT5 and PG-CT6 exhibited their highest values (Figure 6). As ripening progresses, total polyphenol content decreases, probably due to changes such as hydrolysis of glycosides, phenols oxidation and free phenols polymerization (Remorini et al., 2008). The relatively high TPC values measured in pomegranate are in agreement with several authors (Blainski et al., 2013; El Kar et al., 2011; Gil et al., 2000; Ozgen et al., 2008). Furthermore, if compared with other fruit juices, pomegranate juice is characterized by a higher phenolic content which provides antioxidant activity (Dávalos et al., 2005; Calín-Sánchez et al., 2013; Legua et al, 2016).

The antioxidant values of the pomegranate juices (measured for the fruits harvested in 2015), and evaluated with DPPH and ABTS assays, are shown in Table 7. For all juice samples, ABTS values generally decreased in the last harvest date (third week of October), in correspondence with the increase of TAC content (Figure 5); this is probably due to the contribution of phenolic compounds to the biosynthesis of flavylium ring of anthocyanins (Kulkami and Aradhya, 2005). Akko, PG-CT5 and Valenti showed an

increase both for DPPH and ABTS values during the first week of October, while DPPH values of Wonderful and Mollar increased along the maturation.
The Oxygen Radical Absorbance Capacity (ORAC) assay showed some differences between the two years evaluated and especially for some of the tested varieties (Table 8) such as in the case of Valenti and Primosole which, for the second year of observation showed higher ORAC-values as already reported by Todaro et al. (2016). On the whole, the ORAC values of the pomegranate juices are in accordance with those of the recommended database for selected food of USDA (2010).

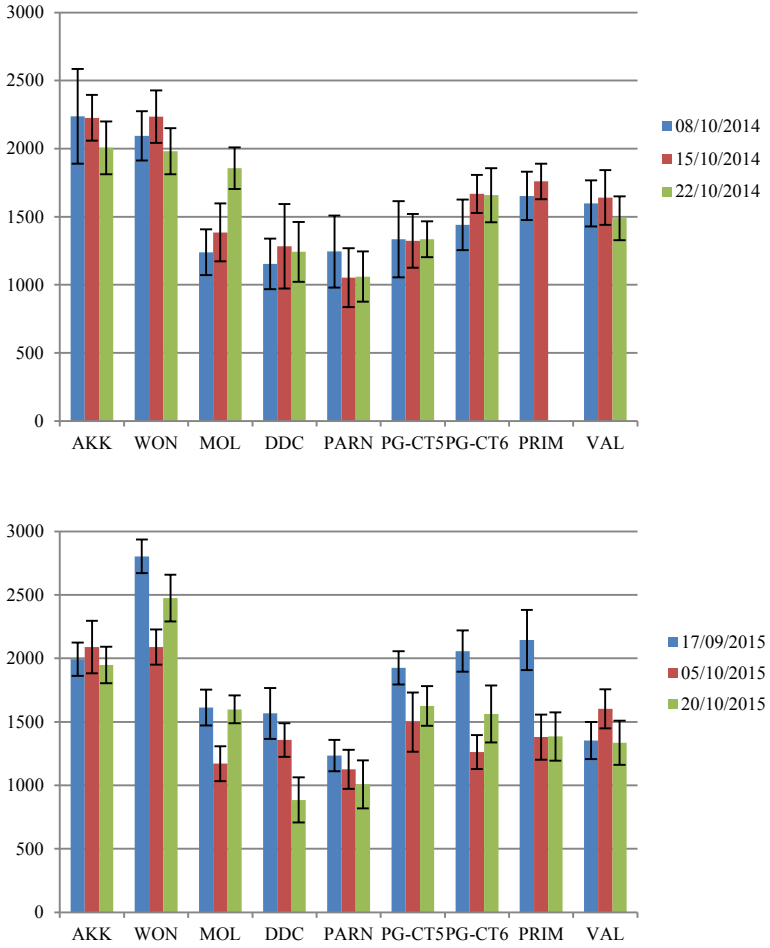


Figure 6. Total polyphenols content (mg GAE L^{-1}) on pomegranate varieties at three harvest times in 2014 (above) and 2015 (below).

Table 7. Antioxidant activity (ABTS+ and DPPH• methods) (mmol TE kg^{-1} FW) on pomegranate varieties at three harvest times in 2015.

	ABTS			DPPH		
	17/09/2015	**05/10/2015**	**20/10/2015**	**17/09/2015**	**05/10/2015**	**20/10/2015**
AKK	7.46±0.32	10.68±0.56	5.99±0.97	8.07±0.16	13.30±0.30	7.58±1.79
WON	3.79±0.41	5.91±0.42	5.46±0.26	5.69±0.28	8.28±0.76	8.64±1.35
MOL	7.74±0.95	3.22±0.87	6.88±1.14	5.95±0.49	5.65±1.67	9.31±0.79
DDC	6.94±0.25	4.91±0.21	3.71±0.59	10.60±1.50	10.38±0.24	3.84±1.23
PARN	4.79±0.46	10.25±0.94	4.21±0.30	8.59±1.07	5.04±0.58	4.58±0.44
PG-CT5	9.39±0.76	8.35±0.67	4.38±0.35	6.23±0.40	10.41±0.22	7.85±0.59
PG-CT6	2.85±0.42	9.63±0.57	7.93±1.18	11.17±0.42	5.12±1.26	7.15±0.68
PRIM	4.90±0.31	3.06±0.62	1.26±0.79	8.59±0.30	8.71±0.37	7.61±2.21
VAL	4.77±1.18	9.45±1.10	1.97±1.12	6.99±0.16	10.74±1.47	7.83±0.20

[a] Mean ± st.dv (Standard deviation)

Table 8. Antioxidant activity (ORAC method) (μmol TE 100 mL^{-1}) measured on pomegranate varieties at three harvest times in 2014 and 2015.

	ORAC					
	08/10/2014	**15/10/2014**	**22/10/2014**	**17/09/2015**	**05/10/2015**	**20/10/2015**
AKK	2122.8a [a]	2844.4a	1981.0a	1263.0bc	2088.0abc	2688.8a
WON	1642.5b	1949.1ab	1589.3b	1032.9c	2539.7ab	2468.6ab
MOL	1299.0cd	1413.43b	1223.5bc	1417.8bc	1279.2cd	1834.1cde
DDC	1150.9d	1399.0b	1381.5bc	1826.7ab	1053.5d	1431.4de
PARN	1133.5d	1158.34b	1339.7bc	1152.0c	1218.2d	1270.3e
PG-CT5	1301.1cd	1818.8ab	1708.0ab	2383.2a	1712.6bcd	2239.4abc
PG-CT6	1616.5bc	2105.0ab	1601.5b	1082.9c	1241.3d	1957.2bcd
PRIM	1125.9d	1743.5ab	1086.4c	1428.3bc	1136.7d	2127.8abc
VAL	1731.0b	1606.4b	1797.9ab	1397.4bc	2859.8a	2382.0abc

[a] Values along columns with different letters are different for P≤0.05

4. Conclusions

In the case of fruit species, and especially for minor fruit species, the local germplasm displays a rather wide range of variability for many important traits, including qualitative ones. Of course this variability must be considered along the years and needs to be validated in different conditions. Taking into account these considerations the results of the present study contribute to a better knowledge of the potential of some accessions of the local Sicilian pomegranate germplasm for which a relevant activity of recognition, propagation and characterization has been performed in the last few years, resulting in the diffusion of some interesting new accessions, such as Primosole (La Malfa et al., 2009).

On the whole the results of this study contribute to the knowledge of the inner characteristics of some of these accessions in comparison with some of the most widespread varieties. Among these, Wonderful juices displayed the higher values of antioxidant activity, total anthocyanin, polyphenol and mainly total acidity content. Although the sweetest varieties and accessions have showed less anthocyanins content, they can be appreciated by consumers for a good TSS:TA ratio, accompanied by a good polyphenol content since the first harvest period.

This study also accomplish to the need of a better characterization of Sicilian pomegranate accessions, allowing to individuate the optimal ripening indexes and parameters and also the optimal harvesting time in order to enlarge the market calendar of such a product.

CONCLUDING REMARKS

The present thesis expands the knowledge on the effect of some agronomical and postharvest factors on the qualitative traits of two important fruit species with a high nutraceutical potential, i.e. citrus and pomegranate.

One of the key objectives of the research deals with the evaluation of the influence of the genotype, including both cultivar and rootstock, on the qualitative profiles of fruits to be used either for fresh consumption or to be processed, with a special emphasis on those bioactive compounds (i.e. polyphenols and anthocyanins) appreciated for their effect on the organoleptic characteristics and for their potential as nutraceutical.

As for citrus, the study of several rootstocks on fruit quality of two pigmented cultivar (Tarocco Scirè and Mandared) has deepen the knowledge of their influence on organoleptic and nutraceutical content, giving also important results about the vegetative and productive characteristics of the different scion/rootstock combinations and on their adaptability in the tested environmental conditions, highlighting some limitations.

On the whole, C35, Bitters, Carpenter and Furr, rootstocks of very recent introduction in Italy, and for which poor knowledge is available, resulted to be the most suitable rootstocks for pigmented oranges and hybrids in the tested conditions and can therefore be proposed as CTV resistant rootstocks. These rootstocks positively affected yield precocity and enhanced fruit pulp anthocyanin content, so allowing to the two different pigmented varieties to display their qualitative potential in terms of anthocyanin

biosynthesis and accumulation in the fruits. Actually these compound are more and more considered for their antioxidant properties. In this work the antioxidant activity of pigmented citrus juice, deriving from an orange and a mandarin hybrid (tangor), was determined with different methods among those available in the literature. Although the tested methods are not fully comparable, as they take into account different classes of free radicals, the results of the *in vitro* tests represent the first contribution about the evaluation of the scion/rootstock combination in pigmented citrus varieties.

As for pomegranate, this represents a minor fruit species, still not fully exploited, at least in Italy, and for which the local germplasm is supposed to display a rather wide range of variability for many important traits, including qualitative ones. For this reason the study herein reported has been focused on the varietal characterization of some Sicilian accessions in comparison with international varieties. Some of the accessions of the local germplasm showed lower anthocyanins content as compared to the most widespread cultivars. Nevertheless, two of these accessions, i.e. Primosole and Valenti, showed a good organoleptic profile accompanied by high ORAC values and polyphenol content, similar to those of the international varieties, and can be considered suitable for fresh consumption. Among the international varieties, Wonderful juices displayed higher values of antioxidant activity, total anthocyanin, polyphenol and mainly total acidity content. This variety represents nowadays somewhat a "standard" for the market. Even though the sweetest varieties and accessions analyzed have showed less anthocyanins content, some of them can be

appreciated by consumers for a good TSS:TA ratio, accompanied by a good polyphenol content and can contribute to widen the offer and also may have a potential for breeding programs. The study in fact allowed to individuate the optimal ripening indexes and parameters and also the optimal harvesting time in order to enlarge the market calendar of such a product.

As a consequence of the recent development of the crop, little information is available for pomegranate about environmental conditions effects on fruit quality parameters and about the maturation evolution of the most widespread varieties cultivated in different areas. In the study on pomegranate cultivars grown in Spain and Italy the cultivation area resulted to be important for the influence on organoleptic and bioactive compounds biosynthesis and mainly on ripening period of each cultivar. The Spanish environment, considered in this experimental plan seems to fasten ripening process and to promote a higher anthocyanins content in the juice.

The possibility to enhance the accumulation of some bioactive compounds in the fruit, several pre and post harvest treatments have been studied for several horticultural species

On the specific of anthocyanins content, in this work some postharvest storage conditions have been evaluated in a late maturing Tarocco line. The cold storage protocols induced a relevant increase in total anthocyanin content, demonstrating that cold treatments of raw fruit can be effective as a useful strategy to guarantee the availability of fresh-high-quality juice, far from the harvest season, suggesting that specific qualitative traits can be further

exploited adopting proper innovative methodologies along the storage and distribution chain.

On the whole the present thesis represents a contribution on different factors involved in the quality assessment of fruit products. Of course, being quality the aptitude of a product in relationship with its use and user, several aspects must be considered, spanning from the requirements of growers, of market operators, up to those of consumers. Each of these operators emphasizes different aspects so that a deep knowledge of the several traits involved in quality concept, along with their evolution, is needed. The results here reported for three important blood citrus varieties and for about ten rootstocks, including some of very new release or introduction, and for a dozen of pomegranate genotypes, evaluated in two different environmental conditions, add valuable knowledge on several aspects related to the quality achievement and management for these species.

REFERENCES

22nd Regional FAO Conference for Europe, Oporto (2000).

Adams, R.P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry (4th ed.). Carol Stream, IL, USA: Allured Publ. Corp.

Agusti, M., Almela, V., Juan, M., Mesejo, C., Martinez-Fuentes, A. (2003). Rootstock influence on the incidence of rind breakdown in Navelate sweet orange. J. Hortic. Sci. Biotech. 78, 554–558.

Ayala-Gil, M.E. and Lozoya, G.E. (2016). Methods for increasing the nutraceutical content of perishable fruits. http://www.freepatentsonline.com/y2016/0235080.html

Albert, Wu G., Prochnik, S., [...], Gmitter, F., Rokhsar, D. (2014). Sequencing of diverse mandarin, pummelo and orange genomes reveals complex history of admixture during citrus domestication. Nature biotechnology 32, 656-663.

Al-Maiman, S.A., Ahmad, D. (2002). Changes in physical and chemical properties during pomegranate (*Punica granatum* L.) fruit maturation. Food Chemistry 76, 437-441.

Alothman, M., Bhat, R., Karim, A.A. (2009). Effects of radiation processing on phytochemicals and antioxidants in plant produce. Trends in Food Science & Technology 20, 201-212.

AOAC (1995). Official methods of analysis (16th ed.). Washington DC: Association of Official Analytical Chemists.

(APHIS-PPQ) Animal and Plant Health Inspection Service - Plant Protection and Quarantine (2006). PPQ Treatment manual. http://www.aphis.usda.gov/ppq/manuals/online_manuals.html (September, 31st, 2006).

Arena, E., Fallico, B., Maccarrone, E. (2001). Evaluation of antioxidant capacity of blood orange juices as influenced by constituents, concentration process and storage. Food Chemistry, 74, 423-427.

Bai, J., Manthey, J. A., Ford, B. L., Luzio, G., Cameron, R. G., Narciso, J., Baldwin, E. A. (2013). Effect of extraction, pasteurization and cold storage on flavonoids and other secondary metabolites in fresh orange juice. Journal of the Science of Food and Agriculture, 93, 2771–2781.

Baldi, S. (2011). GAIN (Global Agricultural Information Network) Report: Italian Citrus Fruit 2010-2011. USDA STAFF, Rome .

Baldwin, E.A. (1993). Citrus fruit. In: Seymour GB, Taylor JE, Tucker GA (eds), Biochemistry of Fruit Ripening, 107-149. Chapman & Hall, London.

Baldwin, E.A., Bai, J., Plotto, A., Cameron, R., Luzio, G., Narciso, J., Manthey, J., Widmer, W., Ford, B.L. (2012). Effect of extraction method on quality of orange juice: hand-squeezed, commercial fresh squeezed and processed.

Journal of the Science of Food and Agriculture, 92, 2029-2042.

Barone, E., Caruso, T., Marra, F.P., Sottile, F. (2001). Preliminary observations on some Sicilian pomegranate (*Punica granatum* L.) varieties. J. Am. Pomol. Soc. 55, 4-7.

Barreca, D., Gattuso, G., Laganà, G., Leuzzi, U., & Bellocco, E. (2016). C- and O-glycosyl flavonoids in Sanguinello and Tarocco blood orange (*Citrus sinensis* (L.) Osbeck) juice: identification and influence on antioxidant properties and acetylcholinesterase activity. Food Chemistry 196, 619- 627.

Barrett, H.C., Rhodes, A.M. (1976). A numerical taxonomic study of affinity relationships in cultivated Citrus and its close relatives. Systematic Botany 1, 105-136.

Belitz, H.-D., Grosch, W., Schieberle, P. (2009). Food Chemistry. 4th revised and extended ed. Springer-Verlag Berlin Heidelberg.

Benzie, I.F.F., Strain, J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. Anal. Biochem. 239, 70-76.

Bhandari, P.R. (2012). Pomegranate (*Punica granatum* L). Ancient seeds for modern cure? Review of potential therapeutic applications. Int. J. Nutr. Pharmacol. Neurol. Dis. 2:171-84.

Blainski, A., Lopes, G.C., Palazzo de Mello, J.C. (2013). Application and analysis of the Folin-Ciocalteu method for

the determination of the total phenolic content from Limonium brasiliense L. Molecules 18, 6852-6865.

Borochov-Neori, H., Judeinstein, S., Tripler, E., Harari, M., Greenberg, A., Shomer, I., Holland, D. (2009). Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit. J. Food Compos. Anal. 22, 189-195.

Borochov-Neori, H., Judeinstein, S., Harari, M., Bar-Ya'akov, I., Patil, B.S., Lurie, S., Holland, D. (2011). Climate effects on anthocyanin accumulation and composition in the pomegranate (*Punica granatum* L.) fruit arils. J. Agric. Food Chem. 59, 5325-5334.

Butelli, E., Licciardello, C., Zhang, Y., Liu, J., Mackay, S., Bailey, P., Reforgiato Recupero, G., Martin, C. (2012). Retrotransposons control fruit specific, cold-dependent accumulation of anthocyanins in blood oranges. Plant Cell 24, 1242-1255.

Calín-Sánchez, A., Martínez-Nicolás, J.J., Munera-Picazo, S., Carbonell-Barrachina, A.A., Legua, P., Hernández, F. (2013). Bioactive compounds and sensory quality of black and white mulberries grown in Spain. Plant Foods Hum Nutr. 68, 370-7.

Cameron, J.W., Soost R.K. (1986). C35 and C32: Citrange rootstocks for citrus. HortScience 21, 157-158.

Cao, G., Alessio, H.M., Cutler, R.G. (1993). Oxygen-radical absorbance capacity assay for antioxidants. Free Radical Biol. Med. 14, 303-311.

Cassano, A., Marchio, M., Drioli, E. (2007). Clarification of blood orange juice by ultrafiltration: analyses of operating parameters, membrane fouling and juice quality. Desalination 212, 15-27.

Castle, W.S. (1995). Rootstock as a fruit quality factor in citrus and deciduous tree crops. New Zealand Journal of Crop and Horticultural Science 23, 383-394.

Castle, W.S. (2010). A career perspective on Citrus rootstocks, their development, and commercialization. HortScience 45, 11-15.

Chapot, H., (1975). The citrus plant. In: Citrus Technical Monograph 4. Ciba-Geigy Agrochemicals, 6-13.

Chao, S., Schreuder, M., Young, G., Nakaoka, K., Moyes, L., and Oberg, C., 2004. Pre-clinical study: Antioxidant levels and immunomodulatory effects of wolfberry juice and other juice mixatures in mice. JANA 7, 32-38.

Clifford, M. N., Zheng, W., Kuhnert, K. (2006). Profiling the chlorogenic acids of Aster by HPLC-MS^n. Phytochemical Analysis 17, 384-393.

Clifford, M. N., Wu, W., Kirkpatrick, J., Kuhnert, N. (2007). Profiling the chlorogenic acids and other caffeic acid derivatives of herbal chrysanthemum by HPLC-MSn. Journal of Agricultural and Food Chemistry 55, 929-936.

Crifò, T., Puglisi I., Petrone, G., Reforgiato Recupero, G., Lo Piero, A.R. (2011). Expression analysis in response to

low temperature stress in blood oranges: implication of the flavonoid biosynthetic pathway. Gene 476, 1-9.

Dávalos, A., Bartolome, B., Gómez-Cordoves, C. (2005). Antioxidant properties of commercial grape juices and vinegars. Food Chemistry 93, 325-330.

Davies, F.S., Albrigo, L.G. (1994). Citrus. In: Crop Production Science in Horticulture Series. CAB International, Wallington.

De la Rosa, L.A., Alvarez-Parrilla, E., Gonzàlez-Aguilar G.A. (2010). Fruit and vegetable phytochemicals: chemistry, nutritional value, and stability. 1th Edition. Wiley J. & Sons, Inc., Publication.

DOGV (2006). Diari Oficial de la Comunitat Valenciana 5346, 30321-30328.

Dugo, P., Mondello, L., Morabito, D., Dugo, G. (2003). Characterization of the Anthocyanin Fraction of Sicilian Blood Orange Juice by Micro-HPLC-ESI/MS. J. Agric. Food Chem. 51, 1173-1176.

El Kar, C., Ferchichi, A., Attia, F., Bouajila, J. (2011). Pomegranate (*Punica granatum*) juices: chemical composition, micronutrient cations, and antioxidant capacity. J. Food Sci. 76, C795-C800.

Engler, A. (1896). Rutaceae. In: Engler, A. and Prantl, K. (eds) Die natürlichen Pflanzenfamilien, Theil 3 Abth. 4, Engelmann, Leipzig.

Erickson, L.C., Brannaman, B.L. (1960). Abscission of reproductive structures and leaves of orange trees. Proc. Am. Soc. Hort. Sci. 75, 222-229.

Fallico, B., Lanza, M. C., Maccarrone, E., Nicolosi Asmundo, C., Rapisarda, P. (1996). Role of hydroxycinnamic acids and vinylphenols in the flavor alteration of blood orange juices. Journal of Agricultural and Food Chemistry 44, 2654-2657.

Fallico, B., Ballistreri, G., Arena, E., Brighina, S., Rapisarda, P. (2017). Bioactive compounds in blood oranges (*Citrus sinensis* (L.) Osbeck): level and intake. Food Chemistry 215, 67-75.

Fang, D.Q., Roose, M.L. (1997). Identification of closely related citrus cultivars with inter-simple sequence repeat markers. Theor. Appl. Genet. 95, 408–417.

FAO (2009) - Food Agriculture Organization of the United Nations. Project document for a regional standard for Pomegranate. Rome, Italy.

Fawole, O.A., Opara, U.L. (2013a). Changes in physical properties, chemical and elemental composition and antioxidant capacity of pomegranate (cv. Ruby) fruit at five maturity stages. Sci. Hortic. 150, 37-46.

Fawole, O.A., Opara, U.L. (2013b). Developmental changes in maturity indices of pomegranate fruit. A descriptive review. Sci. Hortic. 159, 152-161.

Ferrarius, G.B. (1646). Hesperides sive de malorum aureorum cultura et usu libri quatuor. Sumptibus Hermanni Scheus, Roma.

Fischer, U.A., Carle, R., Kammerer, D.R. (2011). Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD−ESI/MSn. Food Chemistry 127, 807-821.

Forner-Giner, M.A., Alcaide, A., Primo-Millo, E., Forner, J.B. (2003). Performance of Navelina orange on 14 rootstocks in Northern Valencia (Spain). Sci. Hortic. 98, 223-232.

Forner-Giner, M.A., Hueso. J.J., Agüera, J.M., Legua. P., Forner. J.B. (2011). Effects of citrus rootstocks on growth, yield and fruit quality of Navelate orange. Journal of Food, Agriculture & Environment 9, 400-403.

Fuleki, T. and Francis, F.J. (1968). Quantitative Methods for Anthocyanins. 4. Determination of Individual Anthocyanins in Cranberry and Cranberry Products. J. Food Sci. 33, 471-478.

Gallesio, G. (1811). Traité du Citrus. Libr. L. Fantin, Paris.

Gattuso, G., Barreca, D., Garguilli, C., Leuzzi, U., Coristi C., 2007. Flavonoid composition of citrus juice. Molecules, 12, 1641-1673.

Gilbert, J., Şenyuva, H.Z. (2008). Bioactive compounds in foods. Black well Publishing Ltd.

Gil, M.I., García-Viguera, C., Artés, F., Tomás-Barberán, F.A. (1995a). Changes in pomegranate juice pigmentation during ripening. J. Sci. Food Agric. 68, 77-81.

Gil, M.I., Cherif, J., Ayed, N., Artés, F., Tomás-Barberán, F.A. (1995b). Influence of cultivar, maturity stage and geographical location on the juice pigmentation of Tunisian pomegranates. Z Lebensm-Unters Forschu 201, 361-364

Gil, M.I., Tomás-Barberán, F.A., Hess-Pierce, B., Holcroft, D.M., Kader, A.A. (2000). Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Composition and Processing. J. Agric. Food Chem. 48, 4581-4589.

Gmitter, F.G. Jr, Xiao, S.Y., Huang, S., Hu, X.L., Garnsey, S.M., Deng, Z. (1996). A localized linkage map of the citrus tristeza virus resistance gene region. Theoretical and Applied Genetics. 92, 688-695.

Goldschmidt, E.E., Monselise, S.P. (1977). Physiological assumptions toward the development of a citrus fruiting model. Proc. Int. Soc. Citricult. 2, 668-672.

Gómez-Caravaca, A.M., Verardo, V., Toselli, M., Segura-Carretero, A., Fernández-Gutiérrez, A., Cabon, M.F. (2013). Determination of the Major Phenolic Compounds in Pomegranate Juices by HPLC−DAD−ESI-MS. J. Agric. Food Chem., 61, 5328-5337.

Goor, A., Liberman J. (1956). The Pomegranate. J. Atsmon (ed.), State of Israel, Ministry of Agriculture, Agr. Publ. Section, Tel Aviv., 5-57.

Grosso, G., Galvano, F., Mistretta, A., Marventano, S., Nolfo, F., Calabrese, G., Buscemi, S., Drago, F., Veronesi, U., Scuderi, A. (2013). Red Orange: Experimental models and epidemiological evidence of its benefits on human health. Oxidative Medicine and Cellular Longevity, 1-11.

Guarino, L., Miller, T., Baazara, M., Obadi, N. (1990). Socotra: The island of Bliss revisited. Diversivity 6, 28-31.

Guerra, D., Schifino Wittmann, M.T., Schwarz, S.F., Dutra de Souza, P.V., Pereira Gonzatto, M., Weiler, R.L. (2014). Comparison between diploid and tetraploid citrus rootstocks: morphological characterization and growth evaluation. Bragantia, Campinas 73, 1-7.

Jahn, O.L. (1973). Inflorescence types and fruiting patterns in 'Hamlin' and 'Valencia' oranges and 'Marsh' grapefruit. Am. J. Bot. 60, 663-670.

Jha, S.N. (2010). Colour measurements and modeling. In: Nondestructive Evaluation of Food Quality, Springer Berlin Heidelberg, 17-40.

Jiménez-Cuesta, M., Cuquerella, J., Martínez-Jávega, J. (1981). Determination of a colour index for citrus fruit degreening. Proceedings of the International Society Citriculture.

Jurenka, J. (2008). Therapeutic Applications of Pomegranate (*Punica granatum* L.): A Review. Altern. Med. Rev. 13, 128-144.

Harlan, J.R. (1992). Crops and man, 2nd ed. Am. Soc. Agronomy and Crop Science Society of America, Madison, WI.

Hamurcu, M., Ozcan, M.M., Dursun, N., Gezgin, S. (2010). Mineral and heavy metal levels of some fruits grown at the roadsides. Food Chem. Toxicol. 48, 1767-1770.

Hasnaoui, N., Mars, M., Ghaffari, S., Trifi, M., Melgarejo, P Hernández, F. (2011). Seed and juice characterization of pomegranate fruits grown in Tunisia: Comparison between sour and sweet cultivars revealed interesting properties for prospective industrial applications. Ind. Crops Prod. 33, 374-381.

Hernández, F., Melgarejo, P., Legua, P., Martínez, R., Martínez, J.J. (2012). Potential correlation between growth habit and yield of Spanish pomegranate cultivars. Sci. Hort. 144, 168-171.

Hermann, P. (1687). Horti Academici Lugduno-Batavi Catalogus. Boutesteyn, Leiden.

Hyoung, S.L. (2002). Characterization of Major Anthocyanins and the Colour of Red-Fleshed Budd Blood Orange (*Citrus sinensis*). J. Agric. Food Chem. 50, 1243-1246.

Hillebrand, S., Schwarz, M., & Winterhalter, P. (2004). Characterization of anthocyanins and pyranoanthocyanins from blood orange (*Citrus sinensis* (L.) Osbeck) juice. Journal of agricultural and Food Chemistry, 52, 7331-7338.

Hodgson, R.W. (1961). Classification and nomenclature of Citrus fruit. California Citrograph, 48.

Holland, D., Hatib, K., Bar-Ya'akov, I. (2009). Pomegranate: botany, horticulture, breeding. Horticultural Reviews 35, 127-192.

Hooker, J.D. (1875). The Flora of British India. 7 Volumes, Reeve & Co., London.

Hutchison, D.J. (1974). Swingle citrumelo - a promising rootstock hybrid. P. Fl. St. Hortic. Soc. 87, 89-91.

Kalra, E.K. (2003). Nutraceutical-definition and introduction. AAPS PharmSci 5, 1-2.

Kasimsetty, S.G., Bialonska, D., Reddy, M.K., Ma, G., Khan, S.I., Ferreira, D. (2010). Colon cancer chemopreventive activities of pomegranate ellagitannins and urolithins. J. Agric. Food Chem. 58, 2180-2187.

Kelebek, H., Canbas, A., Selli, S. (2008). Determination of phenolic composition and antioxidant capacity of blood orange juices obtained from cvs. Moro and Sanguinello (*Citrus sinensis* (L.) Osbeck) grown in Turkey. Food Chemistry 107, 1710-1716.

Khan, I.A. (2007). Citrus genetics, breeding and biotechnology. Ed. CABI.

Köhler, F.E. (1980). *Punica granatum* L. pomegranate. Medizinal Pflanzen 2, 1102.

Kosenko, V.N. (1985). Palynomorphology of representatives of the family Punicaceae. Bot. Z. 70, 39-41.

Kulkarni, A.P., Aradhya, S.M. (2005). Chemical changes and antioxidant activity in pomegranate arils during fruit development. Food Chemistry 93, 319-324.

Kuskoski, E., Asuero, A., Troncoso, A., Mancini-Filho, J., Fett, R. (2005). Aplicación de diversos métodos químicos para determinar actividad antioxidante en pulpa de frutos. Food Science and Technology, Campinas 25, 726-732.

Iglesias, D.J., Cercós, M., Colmenero-Flores, J. M., Naranjo, M.A., Ríos, G., Carrera, E., Ruiz-Rivero, O., Lliso, I., Morillon, R., Tadeo , F.R., Talon, M. (2007). Physiology of citrus fruiting. Braz. J. Plant Physiol. 19, 333-362.

Intrigliolo, F., Reforgiato Recupero, G. (2011). Riconvertire l'agrumicoltura italiana verso una maggiore qualità dell'offerta. Frutticoltura 1/2, 56-57.

IPGRI (2001). Regional report CWANA 1999–2000 (International Plant Genetic Resources Institute, Rome, Italy) <www.ipgri.cgiar.org/publications/pdf/821.pdf>. 1 Sept. 2006.

Lacroix, C.R., Carmentran, M. (2001). Fertilizers and the strawberry plant: yield and fruit quality. Fertilisation du fraisier: rendement et qualité des fruits. Infos Ctifl 170, 41-44.

Ladaniya, M. (2008). Citrus Fruit: Biology, Technology and Evaluation. Academic Press.

Lado, J., Rodrigo, M.J., Zacarías, L. (2014). Maturity indicators and citrus fruit quality. Stewart Postharvest Review, 10, 2:2.

La Malfa, S., Gentile, A., Domina, F., Tribulato, E. (2009). Primosole: A new selection from Sicilian pomegranate germplasm. Acta Hort. 818, 125-132.

Lansky, E.P., Newman, R.A. (2007). Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnophamacol. 109, 177-206.

Lee, H. S. (2002). Characterization of major anthocyanins and the colour of red-fleshed budd blood orange (Citrus sinensis). J. Agric. Food Chem. 50, 1243-1246.

Legua, P., Melgarejo, P., Abdelmajid, H., Martínez, J.J., Martínez, R., Ilham, H., Habida, H., Hernández, F. (2012). Total phenols and antioxidant capacity in 10 Moroccan pomegranate varieties. J. Food Sci. 71, 115-120.

Legua, P., Forner-Giner, M.Á., Nuncio-Jáuregui, N., Hernández F. (2016). Polyphenolic compounds, anthocyanins and antioxidant activity of nineteen pomegranate fruits: A rich source of bioactive compounds. Journal of Functional Foods 23, 628-636.

Linneus, C. (1737). Genera Plantarum. Holmiae, Stokholm.

Liu, Q., Xu, J., Liu, Y., Zhao, X., Deng, X., Guo, L., Gu, J. (2007). A novel bud mutation that confers abnormal patterns

of lycopene accumulation in sweet orange fruit (*Citrus sinensis* L. Osbeck). J. Exp. Bot. 58, 4161-71.

Liu, X., Li, J., Huang, M., Chen, J. (2015). Mechanisms for the influence of citrus rootstocks on fruit size. J. Agric. Food Chem. 63, 2618-27.

Lo Piero, A.R. (2015). The State of the Art in Biosynthesis of Anthocyanins and Its Regulation in Pigmented Sweet Oranges [(*Citrus sinensis*) L. Osbeck.] Journal of Agricultural and Food Chemistry, 63, 16, 4031–4041.

Lo Scalzo, R., Iannoccari, T., Summa, C., Morelli, R., Rapisarda, P. (2004). Effect of thermal treatments on antioxidant and antiradical activity of blood orange juice. Food Chemistry, 85, 41–47.

Louzada, E.S., Del Rio, H.S., Sétamou, M., Watson, J.W., Swietlik, D.M. (2013). Evaluation of citrus rootstocks for the high pH, calcareous soils of South Texas. Euphytica 168, pp.13-18.

Maccarone, E., Maccarone, A., Perrine, G., Rapisarda, P. (1983). Anthocyanins of the Moro orange juice. Ann. Chim. *73*, 533-539.

Maccarone, E., Maccarrone, A., Rapisarda, P. (1985). Acylated anthocyanins from oranges. Ann. Chim., 75, 79-86.

Maccarrone, E., Rapisarda, P., Fanella, F., Arena, A., Mondello, L. (1998). Cyanidin-3-(6’’-malonyl)-β-glucoside.

One of the mayor anthocyanins in blood orange juice. Italian Journal of Food Science, 50, 367-372.

Machado, F.L.C., Costa, J.P., Teixeira, A.S., Costa, J.M.C. (2015). The influence of rootstock and time of harvest on the fruit quality during storage of in two grapefruit cultivars. Acta Sci., Agron. 37, 341-346.

Manera, F.J., Legua, P., Melgarejo, P., Martínez, R., Martínez, J.J., Hernández, F., 2012. Effect of air temperature on rind colour development in pomegranates. Scientia Horticulturae 134, 245-247.

Martínez-Cuenca, M.-R., Primo-Capella, A., Forner-Giner, M.A. (2016). Influence of Rootstock on Citrus Tree Growth: Effects on Photosynthesis and Carbohydrate Distribution, Plant Size, Yield, Fruit Quality, and Dwarfing Genotypes. Plant Growth, Edited by Everlon Cid Rigobelo, Publisher: InTech.

Melgarejo, P., Salazar, D.M., Artés, F. (2000). Organic acids and sugars composition of harvested pomegranate fruits. Eur. Food Res. 211, 185-190.

Melgarejo, P., Salazar, D.M., (2003). Tratado de fruticultura para zonas áridas y semiáridas. 1ª edición, Volumen II. Algarrobo, Granado y Jinjolero. AMV ediciones. Mundi-Prensa.

Melgarejo-Sánchez, P., Martínez, J.J., Legua, P., Martínez, R., Hernández F., Melgarejo, P. (2015). Quality, antioxidant activity and total phenols of six Spanish pomegranates clones. Scientia Horticulturae 182, 65-72.

Mena, P., García-Viguera, C., Navarro-Rico, J., Moreno, D.A., Bartual, J., Saura, D., Marti, N. (2011). Phytochemical characterisation for industrial use of pomegranate (*Punica granatum* L.) cultivars grown in Spain. J. Sci. Food Agric. 91, 1893-1906.

Mphahlele, R.R., Fawole, O.A., Stander, M.A., Opara, U.L. (2014). Preharvest and postharvest factors influencing bioactive compounds in pomegranate (*Punica granatum* L.) -A review. Scientia Horticulturae 178, 114-123.

Mennone, C., Catalano, L. (2014). L'innovazione varietale per la competitività dell'agrumicoltura italiana. Frutticultura 3, 36-43.

Mestre, P.F., Asins, M.J., Pina, J.A., Navarro, L. (1997). Efficient search for new resistant genotypes to the citrus tristeza closterovirus in the orange subfamily Aurantioideae. Theor. Appl. Genet. 95, 1282-1288.

Miguel, M.G., Neves, M.A., Antunes, M.D. (2010). Pomegranate (*Punica granatum* L.): A medicinal plant with myriad biological properties - A short review. J. Med. Plants Res. 4, 2836-2847.

Mirdehghan, S.H., Rahemi, M. (2007). Seasonal changes of mineral nutrients and phenolics in pomegranate (*Punica granatum* L.) fruit. Sci. Hort. 111, 120-127.

Moon, B.W., Lim, S.T., Choi, J.S., Suh, Y.K. (2000). Effects of pre or post harvest application of liquid calcium fertilize manufactured from oyster shell on the calcium concentration and quality in stored 'Niitaka' pear fruits.

Journal of the Korean Society for Horticultural Science 41, 61–64.

Moriguchi, T., Omura, M., Matsuta, N., Kazaki, I. (1987). In vitro adventitious shoot formation from anthers of pomegranate. HortScience 22, 947-948.

Morton, J. (1987). Fruits of warm climates. (Miami, FL).

Nicolosi, E., Deng, Z.N., Gentile, A., La Malfa, S., Continella, G., Tribulato, E. (2000). Citrus phylogeny and genetic origin of important species as investigated by molecular markers. Theor. Appl. Genet. 100, 155-1166.

Nuncio-Jáuregui, N., Munera-Picazo, S., Calín-Sánchez, A., Wojdyło, A., Hernández, F., Carbonell-Barrachina, A.A., (2015). Bioactive compound composition of pomegranate fruits removed during thinning. Journal of Food Composition and Analysis 37, 11-19.

Ozgen, M., Durgac, C. Serce, S., Kaya, C. (2008). Chemical and antioxidant properties of pomegranate cultivars grown in Mediterranean region of Turkey. Food Chem. 111, 703-706.

Özkaya, O., Şener, A., Saridaş, M.A., Ünal, U., Valizadeh, A., Dündar, O. (2015). Influence of Fast Cold Chain and Modified Atmosphere Packaging Storage on Postharvest Quality of Early Season-Harvested Sweet Cherries. Journal of Food Processing and Preservation 39, 2119-2128.

Pallottino, F., Menesatti, P., Lanza, M.C., Strano, M.C., Antonuccia, F., Moresib, M. (2012). Assessment of quality-

assured Tarocco orange fruit sorting rules by combined physicochemical and sensory testing. J. Sci. Food Agric. 93, 1176-1183.

Palma, A., Continella, A., Malfa, S. L., Gentile, A.,D'Aquino, S. (2015). Overall quality of ready-to-eat pomegranate arils processed from cold stored fruit. Postharvest Biology and Technology 109, 1-9.

Pathare, P.B., Opara, U.L., Al-Said, F.A.J. (2013). Colour measurement and analysis in fresh and processed foods: a review. Food and Bioprocess Technology 6, 36-60.

Peleg, H., Naim, M., Rousseff, R. L., Zehavi, U. (1991). Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and grapefruits (*Citrus paradisi*). Journal of the Science of Food and Agriculture 57, 417-426.

Pratella, G.C., Tonini, G., Cessari, A. (1969). Postharvest disease problems of Italian citrus fruit. Proc. 1st Int. Citrus Symp. 3, 1317-1323.

Pergola, M., D'Amico, M., Celano, G., Palese, A.M., Scuderi, A., Di Vita, G., Pappalardo, G., Inglese, P. (2013). Sustainability evaluation of Sicily's lemon and orange production: An energy, economic and environmental analysis. J. Environ. Manage. 128, 674-682.

Proteggente, A.R., Saija A., De Pasquale A., Rice-Evans C.A. (2003). The compositional characterisation and antioxidant activity of fresh juices from sicilian sweet orange (*Citrus sinensis* L. Osbeck) varieties. Free Radic. Res. 37, 681-687.

Ramin, A.-A. and Rezanezhad, A. (2005). Effects of citrus rootstocks on fruit yield and quality of Ruby Red and Marsh grapefruit. Fruits 60, 311–317.

Rapisarda, P., Giuffrida, A. (1992). Anthocyanins level in Italian blood oranges. Proc. Int. Soc. Citr. 3, 1130−1133.

Rapisarda, P., Carollo, G., Fallico, B., Tomaselli, F., Maccarrone, E. (1998). Hydroxycinnamic acid sas markers of Italian blood orange juices. Journal of Agricultural and Food Chemistry 46, 464-470.

Rapisarda, P., Tomaino, A., Lo Cascio, R., Bonina, F., De Pasquale, A., Saija, A. (1999). Antioxidant effectiveness as influenced by phenolic content of fresh orange juice. J. Agric. Food Chem. 47, 4718-4723.

Rapisarda, P., Fanella, F., Maccarone, E. (2000). Reliability of analytical methods for determining anthocyanins in blood orange juice. J. Agric. Food Chem. 48, 2249-2252.

Rapisarda P., Russo G. (2000). Fruit quality of five "Tarocco" selections grown in Italy. Proc. Int. Soc. Citricult. IX, 1149-1153.

Rapisarda, P., Fabroni, S., Peterek, S., Russo, G., Mock, H.-P. (2009). Juice of new citrus hybrids (*Citrus clementina* Hort. ex Tan. x *C. sinensis* L. Osbeck) as a souce of natural antioxidants. Food Chemistry 117, 212-218.

Rawson, A., Patras, A., Tiwari, B.K., Noci, F., Koutchma, T., Brunton, N. (2011). Effect of thermal and non thermal processing technologies on the bioactive content of exotic

fruits and their products: Review of recent advances. Food Research International 44, 1875-1887.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolourization assay. Free Radical Biol. Med. 26, 1231-1237.

Reforgiato Recupero, G., Tribulato, E. (2000). Recent development of Citrus scions and rootstocks in Italy. Proc. Int. Soc. Citricult. IX, 66-68.

Reforgiato Recupero, G., Russo, G. (2001). Innovazione varietale in agrumicoltura. L'Informatore agrario 25, 63-65.

Reforgiato Recupero, G., Russo, G. (2002). La propagazione dell'arancio Tarocco e del clementine Comune. Ital. Hort. 9, 81-84.

Reforgiato Recupero, G., Russo, G., Recupero, S. (2005). New promising Citrus triploid hybrids selected from crosses between monoembryonic diploid female and tetraploid male parents. HortScience 40, 516-520.

Remorini, D., Tavarini, S., Degl'Innocenti, E., Loreti, F., Massai, R., Guidi, L. (2008). Effect of rootstocks and harvesting time on the nutritional quality of peel and flesh of peach fruits. Food Chemistry 110, 361-367.

Rivera-Pastrana, D.M., Gardea, A.A., Yahia, E.M., Martínez-Téllez, M.A., González-Aguilar, G.A. (2014). Effect of UV-C irradiation and low temperature storage on bioactive compounds, antioxidant enzymes and radical

scavenging activity of papaya fruit. J. Food Sci. Technol. 51, 3821-3829.

Savage, E.M., Gardner, F.E. (1965). The origin and history of Troyer and Carrizo citranges. The Citrus Industry 46, 4-7.

Saunt, J. (2000). Citrus varieties on the world. Sinclair International LTD, 11-42.

Seeram, P.N., Schulman, R.N., Heber, D. (2006). Pomegranates. Ancient Roots to modern Medicine. Medicinal and Aromatic plants – Industrial profiles. Taylor and Francis, Boca Raton, FL.

Serrano, M. (2012). Pomegranate fruit ripening: nutritional and bioactive compounds. In: Melgarejo P. (ed.), Valero D. (ed.). II International Symposium on the Pomegranate. Zaragoza: CIHEAM/Universidad Miguel Hernández, p. 165-168. (Options Méditerranéennes: Série A. Séminaires Méditerranéens; n. 103). 2. International Symposium on the Pomegranate, 2011/10/19-21, Madrid (Spain).

Scora, R.W. (1988). Biochemistry, taxonomy and evolution of modern cultivated citrus. Proc. Int. Soc. Citricult. 1, 277–289

Scortichini, M. (1990). Il melograno. Rivista di Frutticoltura *2*, 41-48.

Sheikh, M.K. (2006). The pomegranate. International Book Distributing co.

Shilikina, I.A. (1973). On the xylem anatomy of the genus Punica L. Botanicheskii Zhurnal 58, 1628-1630.

Shwartz, E., Glazer, I., Bar-Ya'akov, I., Matityahu, I., Bar-Ilan, I., Holland, D., Amir, R. (2009). Changes in chemical constituents during the maturation and ripening of two commercially important pomegranate accessions. Food Chemistry 115, 965-973.

Skinner ,M., Hunter, D. (2013). Bioactives in Fruit. Health Benefits and Functional Foods. John Wiley & Sons, Ltd.

Siebert, T., Krueger, R., Kahn, T., Bash, J., Vidalakis, G. (2010). Descriptions of new varieties recently distributed from the Citrus Clonal Protection Program. Citrograph, 20-26.

Silva, E. (2008). Influence of preharvest factors on postharvest quality. In Wholesale success: a farmer's guide to selling, postharvest handling, and packing produce (Midwest edition).

Simon, H.-U., Haj-Yehia, A., Levi-Schaffer, F. (2000). Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis 5, pp. 415-418.

Singleton, V.L., Orthofer, R., & Lamuela-Reventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology, 299, 152–178.

Siracusa, L., Ruberto, G. (2014). Chapter II: Plant polyphenol profiles as a tool for traceability and valuable

support to biodiversity. In Polyphenols in Plants: Isolation, Purification and Extract Preparation (15-33), 1st Edition, Elsevier Books.

Sørensen, H. (1990). Glucosinolates: Structure properties function, in Canola and Rapeseed (ed. F. Shahdi). Van Nostrand, New York, 149-172.

Steerbeck, F. (1682). Citricultura, oftregeringhe der uythemsche boomen te weten oranien. J. Jakobs, Antwerp.

Stover, E., Mercure, E.W. (2007). The Pomegranate: A new look at the fruit of paradise. HortScience *42*, 1088-1092.

Swingle, W.T. (1943). The botany of citrus and its wild relatives of the orange subfamily. In: Webber, H.J. and Batchelor, L.D. (eds) The Citrus Industry. University of California Press, California, 129-474.

Tadeo, F.R., Cercós, M., Colmenero- Flores, J.M., Iglesias, D.J., Naranjo, M.A., Ríos, G., Carrera, E., Ruiz- Rivero, O., Lliso, I., Morillon, R., Ollitrault, P., Talon, M. (2008). Molecular physiology of development and quality of citrus. Adv. Bot. Res. 47, 147-223.

Tanaka, T. (1954). Species problem in Citrus (Revisio aurantiacearum, IX). Japan Society Prom. Sci., Veno, Tokyo.

Tanaka, T. (1961). Contribution to the knowledge of *Citrus* classification. Reports Citrologia 107-114.

Teixeira da Silva, J.A., Rana T.S., Narzary D., Verma N., Meshram, D.T., Ranade, S.A. (2013). Pomegranate biology and biotechnology: A review. Sci. Hortic. 160, 85-107.

Tezcan, F., Gultekin-Ozguven, M., Diken, T., Ozcelik, B., Erim, F.B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. Food Chemistry 115, 873-877.

Thompson, A.K. (2003). Fruit and vegetables: harvesting, handling, and storage. Wiley-Blackwell, 480 pages.

Tolkowsky, S. (1938). Hesperides: a History of the Culture and Use of Citrus Fruits. John Bale, Sons and Curnow Ltd, London.

Tomás-Barberán, F.A. and Clifford, M.N. (2000). Flavanones, chalcones and dihydrochalcones nature, occurrence and dietary burden. Journ. Sci. Food Agric., 80 1073-1080.

Tournefort, J.P. (1700). Institutiones Rei Herbariae Editio Altera. Typ. Reg. Paris.

Tounsi, M. S., Wannes, W. A., Ouerghemmi, I., Jegham, S., Ben Njima, Y., Hamdaoui, G., Zemni, H., Marzouk, B. (2010). Juice components and antioxidant capacity of four Tunisian Citrus varieties. Journal of the Science of Food and Agriculture 91, 142-151.

Torres, A.M., Soost, R.K., Diedenhofen, U. (1978). Leaf isozymes as genetic markers in citrus. Am. J. Bot. 65, 869–881.

Tribulato, E., La Rosa, G. (1994). L'arancio "Tarocco" ed i suoi cloni. Rivista di Frutticoltura, 11, 9-14.

Tribulato, E. and Inglese, P. (2012). Gli agrumi. Collana Coltura&Cultura, Bayer CropScience, 1-608.

Turdieva, M. (2004). Collections of ancient crop at risk in Central Asia. Geneflow '04. International Plant Genetic Resources Institute 2004, Rome, Italy.

Türkyılmaz M., 2013. Anthocyanin and organic acid profiles of pomegranate (*Punica granatum* L.) juices from registered varieties in Turkey. Internat. J. Food Sci. Techn. 48, 2086-2095.

U.S. Department of Agriculture, Agricultural Research Service. 2010. Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods, Release 2. Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/orac

Vavilov, N.I. (1951). The origin, variation, immunity and breeding of cultivated plants (translated from the Russian by K.S. Chester). Chronica Botanica 13, n. 1/6, 1-387.

Velasco, R., Licciardello, C. (2014). A genealogy of the citrus family. Nature Biotechnology 32, 640-642.

Viuda-Martos, M., Fernández-López, J., Pérez-Álvarez, J.A. (2010). Pomegranate and its Many Functional Components as Related to Human Health: A Review. Comprehensive Reviews in Food Science and Food Safety 9:6, 635-654.

Zarbà, A.S., Pulvirenti, G. (2006). The consumption of Sicilian red oranges: implications for firms involved in commercialization. J. Bus. Chem. 3, 22-41.

Zhuo, Zo., Wanpeng, X., Yan, H., Chao, N., Zhiqin, Z. (2016). Antioxidant activity of Citrus fruits. Food Chemistry 196, 88.

Webber, H.J. (1967). History and development of the Citrus industry. In: Reuther, W., Batchelor, L.D. and Webber, H.J. (eds) The Citrus Industry, 2nd edn. University of California Press, California, 1-39.

Wutscher, H.K. (1979). Citrus rootstocks. Horticultural reviews, 230-269.

http://www.fao.org/ag/agn/CDfruits_en/b_contenidos/3_packaging/txt_3/p1_activ1_3.html

http://faostat3.fao.org